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(54) Title: A METHOD FOR THE PROPHYLAXIS AND/OR TREATMENT OF PROLIFERATIVE AND/OR INFLAMMATORY SKIN DISORDERS

(57) Abstract

The present invention relates generally to a method for the prophylaxis and/or treatment of skin disorders, and in particular proliferative and/or inflammatory skin disorders, and to genetic molecules useful for same. The present invention is particularly directed to genetic molecules capable of modulating growth factor interaction with its receptor on epidermal keratinocytes to inhibit, reduce or otherwise decrease stimulation of this layer of cells. The present invention contemplates, in a most preferred embodiment, a method for the prophylaxis and/or treatment of psoriasis.

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A METHOD FOR THE PROPHYLAXIS AND/OR TREATMENT OF PROLIFERATIVE AND/OR INFLAMMATORY SKIN DISORDERS

The present invention relates generally to a method for the prophylaxis and/or treatment of skin disorders, and in particular proliferative and/or inflammatory skin disorders, and to genetic molecules useful for same. The present invention is particularly directed to genetic molecules capable of modulating growth factor interaction with its receptor on epidermal keratinocytes to inhibit, reduce or otherwise decrease stimulation of this layer of cells. The present invention contemplates, in a most preferred embodiment, a method for the prophylaxis and/or treatment of psoriasis.

Bibliographic details of the publications numerically referred to in this specification are collected at the end of the description. Sequence Identity Numbers (SEQ ID NOs.) for the nucleotide sequences referred to in the specification are defined following the bibliography.

Throughout this specification, unless the context requires otherwise, the word "comprise", or variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated element or integer or group of elements or integers but not the exclusion of any other element or integer or group of elements or integers.

Psoriasis and other similar conditions are common and often distressing proliferative and/or inflammatory skin disorders affecting or having the potential to affect a significant proportion of the population. The condition arises from over proliferation of basal keratinocytes in the epidermal layer of the skin associated with inflammation in the underlying dermis. Whilst a range of treatments have been developed, none is completely effective and free of adverse side effects. Although the underlying cause of psoriasis remains elusive, there is some consensus of opinion that the condition arises at least in part from over expression of local growth factors and their interaction with their receptors supporting keratinocyte proliferation *via* keratinocyte receptors which appear to be more abundant during psoriasis.

One important group of growth factors are the dermally-derived insulin-like growth factors (IGFs) which support keratinocyte proliferation. In particular, IGF-I and IGF-II are ubiquitous peptides each with potent mitogenic effects on a broad range of cells. Molecules of the IGF type are also known as "progression factors" promoting "competent" cells through DNA synthesis. The IGFs act through a common receptor known as the Type I or IGF-I receptor, which is tyrosine kinase linked. They are synthesised in mesenchymal tissues, including the dermis, and act on adjacent cells of mesodermal, endodermal or ectodermal origin. The regulation of their synthesis involves growth hormone (GH) in the liver, but is poorly defined in most tissues (1).

Particular proteins, referred to as IGF binding proteins (IGFBPs), appear to be involved in autocrine/paracrine regulation of tissue IGF availability (2). Six IGFBPs have so far been identified. The exact effects of the IGFBPs is not clear and observed effects *in vitro* have been inhibitory or stimulatory depending on the experimental method employed (3). There is some evidence, however, that certain IGFBPs are involved in targeting IGF-I to its cell surface receptor.

Skin, comprising epidermis and underlying dermis, has GH receptors on dermal fiboblasts (4). Fibroblasts synthesize IGF-I as well as IGFBPs-3, -4, -5 and -6 (5) which may be involved in targeting IGF-I to adjacent cells as well as to the overlaying epidermis. The major epidermal cell type, the keratinocyte, does not synthesize IGF-I, but possesses IGF-I receptors and is responsive to IGF-I (6).

It is apparent, therefore, that IGF-I and other growth promoting molecules, are responsible for or at least participate in a range of skin cell activities. In accordance with the present invention, the inventors have established that abberations in the normal functioning of these molecules or abberations in their interaction with their receptors is an important factor in proliferative and/or inflammatory skin disorders. It is proposed, therefore, to target these molecules or other molecules which facilitate their functioning or interaction with their receptors to thereby ameliorate the effects of abberant activity during or leading to skin disease conditions.

Accordingly, one aspect of the present invention contemplates a method for ameliorating the effects of a proliferative and/or inflammatory skin disorder in a mammal, said method comprising contacting the proliferating and/or inflamed skin or skin capable of proliferation and/or inflammation with an effective amount of a nucleic acid molecule or chemical analogue thereof capable of inhibiting or otherwise reducing a growth factor mediated cell proliferation and/or inflammation.

Growth factor mediated cell proliferation and inflammation are also referred to as epidermal hyperplasias and may be mediated by any number of molecules such as but not limited to IGF-I, keratinocyte growth factor (KGF), transforming growth factor-α (TGFα), tumour necrosis factor-α (TNFα), interleukin-1, -4, -6 and 8 (IL-1, IL-4, IL-6 and IL-8, respectively), basic fibroblast growth factor (bFGF) or a combination of one or more of the above. The present invention is particularly described and exemplified with reference to IGF-I and its receptor (IGF-I receptor) and to IGF-I facilitating molecules, IGFBPs, since targeting these molecules according to the methods contemplated herein provides the best results to date. This is done, however, with the understanding that the present invention extends to any growth factor or cytokine-like molecule, a receptor thereof or a facilitating molecule like the IGFBPs involved in skin cell proliferation such as those molecules contemplated above and/or their receptors and/or facilitating molecules therefor.

According to this preferred embodiment, there is provided a method for ameliorating the effects of a proliferative and/or inflammatory skin disorder in a mammal, said method comprising contacting the proliferating and/or inflamed skin or skin capable of proliferation and/or inflammation with an effective amount of a nucleic acid molecule or chemical analogue thereof capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation and/or inflammation.

The present invention is particularly described by psoriasis as the proliferative skin disorder. However, the subject invention extends to a range of proliferative and/or inflammatory skin disorders or epidermal hyperplasias such as but not limited to psoriasis, ichthyosis, pityriasis rubra pilaris ("PRP"), seborrhoea, keloids, keratoses,

neoplasias and scleroderma, warts, benign growths and cancers of the skin.

In a preferred embodiment, therefore, the present invention is directed to a method for ameliorating the effects of psoriasis, said method comprising contacting proliferating skin or skin capable of proliferation with an effective amount of a nucleic acid molecule or chemical analogue thereof capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation.

The present invention extends to any mammal such as but not limited to humans, livestock animals (e.g. horses, sheep, cows, goats, pigs, donkeys), laboratory test animals (e.g. rabbits, mice, guinea pigs), companion animals (e.g. cats, dogs) and captive wild animals. However, the instant invention is particularly directed to proliferative and/or inflammatory skin disorders such as psoriasis in humans.

The aspects of the subject invention instantly contemplated are particularly directed to the topical application of one or more suitable nucleic molecules capable of inhibiting, reducing or otherwise interfering with IGF-mediated cell proliferation and/or inflammation. More particularly, the nucleic acid molecule targets IGF-I interaction with its receptor. Conveniently, therefore, the nucleic acid molecule is an antagonist of IGF-I interaction with its receptor. Most conveniently, the nucleic acid molecule antagonist is an antisense molecule to the IGF-I receptor, to IGF-I itself or to a molecule capable of facilitating IGF-I interaction with its receptor such as but not limited to an IGFBP.

Insofar as the invention relates to IGFBPs, the preferred molecules are IGFBP-2, -3, -4, -5 and -6. The most preferred molecules are IGFBP-2 and IGFBP-3.

The nucleotide sequences of IGFBP-2 and IGFBP-3 are set forth in Figures 1 (SEQ ID NO. 1) and 2 (SEQ ID NO. 2), respectively. According to a particularly preferred aspect of the present invention, there is provided a nucleic acid molecule comprising at least about ten nucleotides capable of hybridising to, forming a heterodouplex or otherwise interacting with an mRNA molecule directed from a gene corresponding to

a genomic form of SEQ ID NO. 1 and/or SEQ ID NO. 2 and which thereby reduces or inhibits translation of said mRNA molecule. Preferably, the nucleic acid molecule is at least about 15 nucleotides in length and more preferably at least about 20-25 nucleotides in length. However, the instant invention extends to any length nucleic acid molecule including a molecule of 100-200 nucleotides in length to correspond to the full length of or near full length of the subject genes.

The nucleotide sequence of the antisense molecules may correspond exactly to a region or portion of SEQ ID NO. 1 or SEQ ID NO. 2 or may differ by one or more nucleotide substitutions, deletions and/or additions. It is a requirement, however, that the nucleic acid molecule interact with an mRNA molecule to thereby reduce its translation into active protein.

Examples of potential antisense molecules for IGFBP-2 and IGFBP-3 are those capable of interacting with sequences selected from the lists in Examples 6 and 7, respectively.

The nucleic acid molecules in the form of an antisense molecule may be linear or covalently closed circular and single stranded or partially double stranded. A double stranded molecule may form a triplex with target mRNA or a target gene. The molecule may also be protected from, for example, nucleases, by any number of means such as using a nonionic backbone or a phosphorothioate linkage. A convenient nonionic backbone contemplated herein is ethylphosphotriester linkage or a 2'-O-methylribosyl derivative.

Examples of suitable oligonucleotide analogues are conveniently described in Ts'O *et al* (7).

Alternatively, the antisense molecules of the present invention may target the IGF-I gene itself or its receptor or a multivalent antisense molecule may be constructed or separate molecules administered which target at least two or an IGFBP, IGF-I and/or IGF-I-receptor. Examples of suitable antisense molecules capable of targetting the IGF-I receptor are those capable of interacting with sequences selected from the list in

Example 8. One particularly useful antisense molecule is 5'- ATCTCTCCGCTTCCTTTC -3' (SEQ ID NO. 10). A particularly preferred embodiment of the present invention contemplates a method of ameliorating the effects of psoriasis, said method comprising contacting proliferating skin or skin capable of proliferation with an effective amount of one or more nucleic acid molecules or chemical analogues thereof capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation wherein said one or more molecules comprises a polynucleotide capable of interacting with mRNA directed from two or more of an IGF-I gene, an IGF-I receptor gene or a gene encoding an IGFBP such as IGFBP-2 and/or IGFBP-3.

In accordance with one aspect of the present invention the nucleic acid molecule is topically applied in aqueous solution or in conjunction with a cream, ointment, oil or other suitable carrier and/or diluent. A single application may be sufficient depending on the severity or exigencies of the condition although more commonly, multiple applications are required ranging from hourly, multi-hourly, daily, multi-daily, weekly or monthly, or in some other suitable time interval. The treatment might comprise solely the application of the nucleic acid molecule or this may be applied in conjunction with other treatments for the skin proliferation and/or inflammatory disorder being treated or for other associated conditions including microbial infection, bleeding and the formation of a variety of rashes.

As an alternative to or in conjunction with antisense therapy, the subject invention extends to the nucleic acid molecule as, or incorporating, a ribozyme including a minizyme to, for example, IGF-I, its receptor or to molecules such as IGFBPs and in particular IGFBP-2 and -3. Ribozymes are synthetic nucleic acid molecules which possess highly specific endoribonuclease activity. In particular, they comprise a hybridising region which is complementary in nucleotide sequence to at least part of a target RNA. Ribozymes are well described by Haseloff and Gerlach (8) and in International Patent Application No. WO 89/05852. The present invention extends to ribozymes which target mRNA specified by genes encoding IGF-I, its receptor or one or more IGFBPs such as IGFBP-2 and/or IGFBP-3.

According to this embodiment, there is provided in a particularly preferred aspect a ribozyme comprising a hybridising region and a catalytic region wherein the hybridising region is capable of hybridising to at least part of a target mRNA sequence transcribed from a genomic gene corresponding to SEQ ID NO. 1 or SEQ ID NO. 2 wherein said catalytic domain is capable of cleaving said target mRNA sequence to reduce or inhibit IGF-I mediated cell proliferation and/or inflammation.

Yet another aspect of the present invention contemplates co-suppression to reduce expression or to inhibit translation of an endogenous gene encoding, for example, IGF-I, its receptor, or IGFBPs such as IGFBP-2 and/or -3. In co-suppression, a second copy of an endogenous gene or a substantially similar copy or analogue of an endogenous gene is introduced into a cell following topical administration. As with antisense molecules, nucleic acid molecules defining a ribozyme or nucleic acid molecules useful in co-suppression may first be protected such as by using a nonionic backbone.

The efficacy of the nucleic acid molecules of the present invention can be conveniently tested and screened using an *in vitro* system comprising a basal keratinocyte cell line. A particularly useful system comprises the HaCaT cell line described by Boukamp *et al* (9). In one assay, IGF-I is added to an oligonucleotide treated HaCaT cell line. Alternatively, growth of oligonucleotide treated HaCaT cells is observed on a feeder layer of irradiated 3T3 fibroblasts. Using such *in vitro* assays, it is observed that antisense oligonucleotides to IGFBP-3, for example, inhibit production of IGFBP-3 by HaCaT cells. Other suitable animal models include the nude mouse/human skin graft model (15; 16) and the "flaky skin" mouse model (17; 18). In the nude mouse model, microdermatome biopsies of psoriasis lesions are taken under local anaesthetic from volunteers then transplanted to congenital athymic (nude) mice. These transplanted human skin grafts maintain the characteristic hyperproliferating epidermis for 6-8 weeks. They are an established model for testing the efficacy of topically applied therapies for psoriasis. In the "flaky skin" mouse model, the fsn/fsn mutation produces mice with skin resembling human psoriasis. This mouse, or another mutant mouse with a similar phenotype is a further *in vivo* model to test the efficacy of topically applied therapies for psoriasis.

Another aspect of the present invention contemplates a pharmaceutical composition for topical administration which comprises a nucleic acid molecule capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation such as psoriasis and one or more pharmaceutically acceptable carriers and/or diluents. Preferably, the nucleic acid molecule is an antisense molecule to IGF-I, the IGF-I receptor or an IGFBP such as IGFBP-2 and/or IGFBP-3 or comprises a ribozyme to one or more of these targets or is a molecule suitable for co-suppression of one or more of these targets. The composition may comprise a single species of a nuleic acid molecule capable of targeting one of IGF-I, its receptor or an IGFBP, such as IGFBP-2 or IGFBP-3 or may be a multi-valent molecule capable of targeting two or more of IGF-I, its receptor or an IGFBP, such as IGFBP-2 and/or IGFBP-3.

The nucleic acid molecules may be administered in dispersions prepared in creams, ointments, oil or other suitable carrier and/or diluent such as glycerol, liquid polyethylene glycols and/or mixtures thereof. Under ordinary conditions of storage and use, these preparations may contain a preservative to prevent the growth of microorganisms.

The pharmaceutical forms suitable for topical use include sterile aqueous solutions (where water soluble) or dispersions and powders for the extemporaneous preparation of topical solutions or dispersion. In all cases, the form is preferably sterile although this is not an absolute requirement and is stable under the conditions of manufacture and storage. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof and vegetable oils. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of superfactants. The prevention of the action of microorganism can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thirmerosal and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride.

Topical solutions are prepared by incorporating the nucleic acid molecule compound in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by where necessary filter sterilization.

As used herein "pharmaceutically acceptable carriers and/or diluents" include any and all solvents, dispersion media, aqueous solutions, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like. The use of such media and agents for pharmaceutical active substances is well known in the art. Except insofar as any conventional media or agent is incompatable with the active ingredient, use thereof in the pharmaceutical compositions is contemplated. Supplementary active ingredients can also be incorporated into the compositions. Conveniently, the nucleic acid molecules of the present invention are stored in freeze-dried form and are reconstituted prior to use.

Yet another aspect of the present invention contemplates the use of a nucleic acid molecule in the manufacture of a medicament for the treatment of proliferative and/or inflammatory skin disorders mediated by a growth factor. The proliferative and/or inflammatory skin disorder is generally psoriasis and the nucleic acid molecule targets IGF-I, the IGF-I receptor and/or an IGFBP such as IGFBP-2 and/or IGFBP-3.

Still a further aspect of the present invention contemplates an agent comprising a nucleic acid molecule as hereinbefore defined useful in the treatment of proliferative and/or inflammatory skin disorders, such as psoriasis.

The present invention is further described by the following non-limiting Figures and/or Examples.

In the Figures:

Figure 1 is a representation of the nucleotide sequence of IGFBP-2.

```
LOCUS       HSIGFBP2     1433 bp    RNA             PRI       31-JAN-1990
DEFINITION  Human mRNA for insulin-like growth factor binding protein (IGFBP-2)
ACCESSION   X16302
KEYWORDS    insulin-like growth factor binding protein.
SOURCE      human
  ORGANISM  Homo sapiens
            Eukaryota; Animalia; Metazoa; Chordata; Vertebrata; Mammalia;
            Theria; Eutheria; Primates; Haplorhini; Catarrhini; Hominidae.
REFERENCE   1  (bases 1 to 1433)
  AUTHORS   Binkert,C., Landwehr,J., Mary,J.L., Schwander,J. and Heinrich,G.
  TITLE     Cloning, sequence analysis and expression of a cDNA encoding a
            novel insulin-like growth factor binding protein (IGFBP-2)
  JOURNAL   EMBO J. 8, 2497-2502 (1989)
  STANDARD  full automatic
COMMENT     NCBI gi: 33009
FEATURES             Location/Qualifiers
     source          1. .1433
                     /organism="Homo sapiens"
                     /dev_stage="fetal"
                     /tissue_type="liver"
     misc_feature    1416. .1420
                     /note="pot. polyadenylation signal"
     polyA_site      1433
                     /note="polyadenylation site"
     CDS             118. .1104
                     /note="precursor polypeptide;  (AA -39 to 289);  NCBI gi:
                     33010."
                     /codon_start=1
                     /translation="MLPRVGCPALPLPPPPLLPLLPLLLLLLGASGGGGGARAEVLFR
                     CPPCTPERLAACGPPPVAPPAAVAAVAGGARMPCAELVREPGCGCCSVCARLEGEACG
                     VYTPRCGQGLRCYPHPGSELPLQALVMGEGTCEKRRDAEYGASPEQVADNGDDHSEGG
                     LVENHVDSTMNMLGGGGSAGRKPLKSGMKELAVFREKVTEQHRQMGKGGKHHLGLEEP
                     KKLRPPPARTPCQQELDQVLERISTMRLPDERGPLEHLYSLHIPNCDKHGLYNLKQCK
                     MSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNEQQEACGVHTQRMQ"
     CDS             118. .234
                     /note="signal peptide;  (AA -39 to -1);  NCBI gi: 33011."
                     /codon_start=1
                     /translation="MLPRVGCPALPLPPPPLLPLLPLLLLLLGASGGGGGARA"
     CDS             235. .1101
                     /note="mature IGFBP-2;  (AA 1 to 289);  NCBI gi: 33012."
                     /codon_start=1
                     /translation="EVLFRCPPCTPERLAACGPPPVAPPAAVAAVAGGARMPCAELVR
                     EPGCGCCSVCARLEGEACGVYTPRCGQGLRCYPHPGSELPLQALVMGEGTCEKRRDAE
                     YGASPEQVADNGDDHSEGGLVENHVDSTMNMLGGGGSAGRKPLKSGMKELAVFREKVT
                     EQHRQMGKGGKHHLGLEEPKKLRPPPARTPCQQELDQVLERISTMRLPDERGPLEHLY
                     SLHIPNCDKHGLYNLKQCKMSLNGQRGECWCVNPNTGKLIQGAPTIRGDPECHLFYNE
                     QQEACGVHTQRMQ"
BASE COUNT      239 a    466 c    501 g    227 t
ORIGIN

HSIGFBP2  Length: 1433  May 11, 1994  10:06  Type: N  Check: 6232  ..
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Figure 2 is a representation of the nucleotide sequence of IGFBP-3.

```
LOCUS       HUMGFIBPA    2474 bp ss-mRNA            PRI       15-JUN-1990
DEFINITION  Human growth hormone-dependent insulin-like growth factor-binding
            protein mRNA, complete cds.
ACCESSION   M31159
KEYWORDS    insulin-like growth factor binding protein.
SOURCE      Human plasma, cDNA to mRNA, clone BP-53.
  ORGANISM  Homo sapiens
            Eukaryota; Animalia; Chordata; Vertebrata; Mammalia; Theria;
            Eutheria; Primates; Haplorhini; Catarrhini; Hominidae.
REFERENCE   1  (bases 1 to 2474)
  AUTHORS   Wood,W.I., Cachianes,G., Henzel,W.J., Winslow,G.A., Spencer,S.A.,
            Hellmiss,R., Martin,J.L. and Baxter,R.C.
  TITLE     Cloning and expression of the growth hormone-dependent insulin-like
            growth factor-binding protein
  JOURNAL   Mol. Endocrinol. 2, 1176-1185 (1988)
  STANDARD  full automatic
COMMENT     NCBI gi: 183115
FEATURES             Location/Qualifiers
     mRNA            <1. .2474
                     /note="GFIBP mRNA"
     CDS             110. .985
                     /gene="IGFBP1"
                     /note="insulin-like growth factor-binding protein; NCBI
                     gi: 183116."
                     /codon_start=1
                     /translation="MQRARPTLWAAALTLLVLLRGPPVARAGASSGGLGPVVRCEPCD
                     ARALAQCAPPPAVCAELVREPGCGCCLTCALSEGQPCGIYTERCGSGLRCQPSPDKAR
                     PLQALLDGRGLCVNASAVSRLRAYLLPAPPAPGNASESEEDRSAGSVESPSVSSTHRV
                     SDPKFHPLHSKIIIIKKGHAKDSQRYKVDYESQSTDTQNFSSESKRETEYGPCRREME
                     DTLNHLKFLNVLSPRGVHIPNCDKKGFYKKKQCRPSKGRKRGFCWCVDKYGQPLPGYT
                     TKGKEDVHCYSMQSK"
     source          1. .2474
                     /organism="Homo sapiens"
BASE COUNT      597 a    646 c    651 g    580 t
ORIGIN

HUMGFIBPA  Length: 2474  May 11, 1994  10:00  Type: N  Check: 9946  ..
```

Figure 3 is a representation of the nucleotide sequence of IGF-1-receptor.

```
LOCUS       HSIGFIRR     4989 bp    RNA             PRI       28-MAR-1991
DEFINITION  Human mRNA for insulin-like growth factor I receptor
ACCESSION   X04434 M24599
KEYWORDS    glycoprotein; insulin receptor;
            insulin-like growth factor I receptor; membrane glycoprotein;
            receptor; tyrosine kinase.
SOURCE      human
  ORGANISM  Homo sapiens
            Eukaryota; Animalia; Metazoa; Chordata; Vertebrata; Mammalia;
            Theria; Eutheria; Primates; Haplorhini; Catarrhini; Hominidae.
REFERENCE   1  (bases 1 to 4989)
  AUTHORS   Ullrich,A., Gray,A., Tam,A.W., Yang-Feng,T., Tsubokawa,M.,
            Collins,C., Henzel,W., Bon,T.L., Kathuria,S., Chen,E., Jakobs,S.,
            Francke,U., Ramachandran,J. and Fujita-Yamaguchi,Y.
  TITLE     Insulin-like growth factor I receptor primary structure: comparison
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            with insulin receptor suggests structural dererminants that define
            functional specificity
  JOURNAL   EMBO J. 5, 2503-2512 (1986)
  STANDARD  full automatic
COMMENT     NCBI gi: 33058
FEATURES             Location/Qualifiers
     source          1. .4989
                     /organism="Homo sapiens"
                     /tissue_type="placenta"
                     /clone_lib="(lamda)gt10"
                     /clone="(lambda)IGF-1-R.85, (lambda)IGF-1-R.76"
     sig_peptide     32. .121
     mat_peptide     122. .4132
                     /note="IGF-I receptor"
     misc_feature    122. .2251
                     /note="alpha-subunit (AA 1 - 710)"
     misc_feature    182. .190
                     /note="pot.N-linked glycosylation site (AA 21 - 23)"
     misc_feature    335. .343
                     /note="pot.N-linked glycostlation site (AA 72 - 74)"
     misc_feature    434. .442
                     /note="pot.N-linked glycostlation site (AA 105 - 107)"
     misc_feature    761. .769
                     /note="pot.N-linked glycostlation site (AA 214 - 216)"
     misc_feature    971. .979
                     /note="pot.N-linked glycostlation site (AA 284 - 286)"
     misc_feature    1280. .1288
                     /note="pot.N-linked glycostlation site (AA 387 - 389)"
     misc_feature    1343. .1351
                     /note="pot.N-linked glycosylation site (AA 408 - 410)"
     misc_feature    1631. .1639
                     /note="pot.N-linked glycostlation site (AA 504 - 506)"
     misc_feature    1850. .1858
                     /note="pot.N-linked glycosylation site (AA 577 - 579)"
     misc_feature    1895. .1903
                     /note="pot.N-linked glycosylation site (AA 592 - 594)"
     misc_feature    1949. .1957
                     /note="pot.N-linked glycosylation site (AA 610 - 612)"
     misc_feature    2240. .2251
                     /note="putative proreceptor processing site (AA 707 -
                     710)"
     misc_feature    2252. .4132
                     /note="beta-subunit (AA 711 - 1337)"
     misc_feature    2270. .2278
                     /note="pot.N-linked glycosylation site (AA 717 - 719)"
     misc_feature    2297. .2305
                     /note="pot.N-linked glycosylation site (AA 726 - 728)"
     misc_feature    2321. .2329
                     /note="pot.N-linked glycosylation site (AA 734 - 736)"
     misc_feature    2729. .2737
                     /note="pot.N-linked glycosylation site (AA 870 - 872)"
     misc_feature    2768. .2776
                     /note="pot.N-linked glycosylation site (AA 883 - 885)"
     misc_feature    2837. .2908
                     /note="transmembrane region (AA 906 - 929)"
     misc_feature    2918. .2926
                     /note="pot.N-linked glycosylation site (AA 933 - 935)"
     misc_feature    3047. .3049
                     /note="pot.ATP binding site (AA 976)"
     misc_feature    3053. .3055
                     /note="pot.ATP binding site (AA 978)"
     misc_feature    3062. .3064
                     /note="pot.ATP binding site (AA 981)"
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     misc_feature    3128. .3130
                     /note="pot.ATP binding site (AA 1003)"
     CDS             32. .4132
                     /product="IGF-I receptor"
                     /note="50 stops when translation attempted, frame 1, code
                     0"
BASE COUNT      1216 a   1371 c   1320 g   1082 t
ORIGIN

HSIGFIRR  Length: 4989  May 11, 1994  12:10  Type: N  Check: 133  ..
```

Figure 4A is a photographic representation of a Western ligand blot of HaCaT conditioned medium showing IGFBP-3 secreted in 24 hours after 7 day treatment with phosphorothioate oligonucleotides (BP3AS2, BP3AS3 and BP3S) at 0.5μM and 5μM; * no oligonucleotide added.

Figure 4B is a graphical representation of a scanning imaging desitometry of Western ligand blot (Figure 4A), showing relative band intensities of IGFBP-3 and the 24kDa IGFBP-4 after treatment with phosphorothioate oligonucleotides;
* no oligonucleotide added.

Figure 5A is a photographic representation of a Western ligand blot of HaCaT conditioned medium showing IGFBP-3 secreted in 24 hours after 7 day treatment with phosophorothioate oligonucleotide BP3AS2 at 0.5μM compared with several control oligonucleotides at 0.5μM. (a) oligonucleotide BP3AS2NS; (b) oligonucleotide BP3AS4; (c) oligonucleotide BP3AS4NS; and (untreated), no oligonucleotide added.

Figure 5B is a graphical representation of a scanning imaging densitometry of Western ligand blot (Figure 5A), showing relative band intensities of IGFBP-3 after treatment with phosphorothioate oligonucleotides as in Figure 5A, showing IGFBP-3 band intensities expressed as a percentage of the average band intensity from conditioned medium of cells not treated with oligonucleotide.

Figure 6 is a graphical representation showing inhibition of IGF-I binding by antisense oligonucleotides to IGF-I receptor. IGFR.AS: antisense; IGFR.S: sense.

Figure 7 is a graphical representation showing inhibition of IGFBP-3 production in culture medium following initial treatment with antisense oligonucleotides once daily over a 2 day period.

Figure 8 is a graphical representation showing optimization of IGFBP-3 antisense oligonucleotide concentration as determined by relative IGFBP-3 concentration in culture medium.

EXAMPLE 1

IN VITRO ASSAY: CELLS

The differentiated human keratinocyte cell line, HaCaT (9) was used in the *in vitro* assay. Cells at passage numbers 33 to 36 were maintained as monolayer cultures in 5% v/v CO_2 at 37°C in Keratinocyte-SFM (Gibco) containing EGF and bovine pituitary extract as supplied. Media containing foetal calf serum were avoided because of the high content of IGF-I binding proteins in serum.

Feeder layer plates of lethally irradiated 3T3 fibroblasts were prepared exactly as described by Rheinwald and Green (10).

EXAMPLE 2

IN VITRO ASSAY: THYMIDINE INCORPORATION ASSAY

Cells were grown to 4 days post confluence in $2cm^2$ wells with daily medium changes of Keratinocyte-SFM, then the medium was changed to DMEM (Cytosystems, Australia), with the following additions: 25mM Hepes, 0.19% w/v, sodium bicarbonate, 0.03% w/v glutamine (Sigma Chemical Co, USA), 50IU/ml penicillin and 50µg/ml streptomycin (Flow Laboratories). After 24 hours, IGF-I or tIGF-I was added to triplicate wells, at the concentrations indicated, in 0.5ml fresh DMEM containing 0.02% v/v bovine serum albumin (Sigma molecular biology grade) and incubated for a further 21 hours. [^{3}H]-Thymidine (0.1µCi/well) was then added and the cells incubated for a further 3 hours. The medium was then aspirated and the cells washed once with ice-cold PBS and twice with ice-cold 10% v/v TCA. The TCA-precipitated monolayers were

then solubilized with 0.25M NaOH (200µl/well), transferred to scintillation vials and radioactivity determined by liquid scintillation counting (Pharmacia Wallac 1410 liquid scintillation counter).

EXAMPLE 3

WESTERN LIGAND BLOTTING

HaCaT conditioned medium (250µl) was concentrated by adding 750µl cold ethanol, incubating at -20°C for 2 hours and centrifuging at 16,000g for 20 min at 4°C. The resulting pellet was air dried, resuspended thoroughly in non-reducing Laemmli sample buffer, heated to 90°C for 5 minutes and separated on 12% w/v SDS-PAGE according to the method of Laemmli (1970). Separated proteins were electrophoretically transferred to nitrocellulose membrane (0.45mm, Schleicher and Schuell, Dassel, Germany) in a buffer containing 25mM Tris, 192mM glycine and 20% v/v methanol. IGFBPs were then visualised by the procedure of Hossenlopp *et al* (11), using [^{125}I]-IGF-I, followed by autoradiography. Autoradiographs were scanned in a BioRad Model GS-670 Imaging Densitometer and band densities were determined using the Molecular Analyst program.

EXAMPLE 4

ANTISENSE OLIGONUCLEOTIDES

Phosphorothioate oligodeoxynucleotides were synthesised by Bresatec, Adelaide, South Australia, Australia. The following antisense sequences were used: BP3AS2, 5'- GCG CCC GCT GCA TGA CGC CTG CAA C -3' (SEQ ID NO. 4), a 25mer complementary to the start codon region of the human IGFBP-3 mRNA; BP3AS3, 5'- CGG GCG GCT CAC CTG GAG CTG GCG -3' (SEQ ID NO. 5), a 24mer complementary to the exon 1/intron 1 splice site; BP3AS4, 5'- AGG CGG CTG ACG GCA CTA -3' (SEQ ID NO. 6), an 18mer complementary to a region of the coding sequence lacking RNA secondary structure and oligonucleotide-dimer formation (using the computer software "OLIGO for PC"). Since BP3AS4 was found to be ineffective at inhibiting IGFBP-3 synthesis, it was used as a control. The following additional control oligonucleotide sequences were used: BP3S, 5'- CAG GCG TCA TGC AGC GGG C -3' (SEQ ID NO. 7), an 18mer sense control sequence equivalent to the start codon region; BP3AS2NS, 5'- CGG AGA

TGC CGC ATG CCA GCG CAG G -3' (SEQ ID NO. 8), a 25mer randomised sequence with the same GC content as BP3AS2; BP3AS4NS, 5'- GAC AGC GTC GGA GCG ATC -3' (SEQ ID NO. 9), an 18mer randomised sequence with the same GC content as BP3AS4NS. Design of the oligonucleotides was based on the human IGFBP-3 cDNA sequence of Spratt *et al* (12).

Cells were grown to one day post confluence in $2cm^2$ wells with daily medium changes of 0.5ml Keratinocyte-SFM, then subjected to daily medium changes of Keratinocyte-SFM for a further 4 days. Daily additions of 0.5ml fresh Keratinocyte-SFM were then continued for a further 7 days, except that at the time of medium addition, 5µl oligonucleotide in PBS was added to give the final concentrations indicated, then the wells were shaken to mix the oligonucleotide. After the final addition, cells were incubated for 24 hours and the medium collected for assay of IGFBPs. Cells were then counted after trypsinisation in a Coulter Industrial D Counter, Coulter Bedfordshire, UK. Cell numbers after oligonucleotide treatment differed by less than 10%.

EXAMPLE 5

ANTISENSE OLIGONUCLEOTIDES INHIBIT IGFBP-3 SYNTHESIS

HaCaT cells secrete mainly IGFBP-3 (>95%), with the only other IGFBP detectable in HaCaT conditioned medium being IGFBP-4 (<5%). The effect on IGFBP-3 and IGFBP-4 synthesis of antisense oligonucleotides at two concentrations, 5µM and 0.5µM, was tested. Two oligonucleotides were used, BP3AS2 and BP3AS3, directed against the start site and the intron 1/exon 1 splice site, respectively of the IGFBP-3 mRNA. As a control, a sense oligonucleotide corresponding to the start site was used. As shown in Figures 4A and 4B, all oligonucleotides at 5µM caused a significant reduction of IGFBP-3 synthesis compared with untreated cells, however, the two antisense oligonucleotides inhibited IGFBP-3 synthesis of approximately 50% compared to the sense control (Figure 4B). The antisense oligonucleotide directed to the start codon appeared to be more effective of the two, the difference being more apparent at the lower concentration of 0.5µM. The cells of IGFBP-4 secreted by the HaCaT cells make photographic reproduction of the bands on Western ligand blots difficult, however densitometry measurements provide adequate relative quantitation. This resulted in the

significant observation that IGFBP-4 levels were unaffected by oligonucleotide addition to the cells, suggesting that the observed inhibitory effects on IGFBP-3 are specific.

To further investigate the inhibitory effects of the more effective of the two antisense oligonucleotides, BP3AS2, inhibition by this oligonucleotide at 0.5μM was compared with a number of control oligonucleotides, including one antisense oligonucleotide to IGFBP-3 that had proved to be ineffective at 0.5μM. As shown in Figures 5A and 5B, BP3AS2 was again inhibitory, resulting in levels of IGFBP-3 of approximately 50% of the most non-specifically inhibitory control oligonucleotide, the randomised equivalent of BP3AS2. The other control oligonucleotides caused no reduction in IGFBP-3 levels at 0.5μM, compared to untreated cells. Of possible significance is the fact that this control oligonucleotide, BP3AS2NS, like BP3AS2 itself, has the highest potential T_m of the three control oligonucleotides used in this experiment, enhancing the probability of non-specific base pairing with non-target mRNAs. However, the lack of inhibition of IGFBP-4 secretion by BP3AS2 suggests that this oligonucleotide is selective even compared with the most closely related protein likely to be present in this cell line.

EXAMPLE 6

ANTISENSE OLIGONUCLEOTIDES OF IGFBP2

Antisense oligonucleotides to IGFBP2 may be selected from molecules capable of interacting with one or more of the following sense oligonucleotides:

ATTCGGGGCGAGGGA	AAGAAGCGGAGGAGG	CCCGCTCGCAGGGCC
TTCGGGGCGAGGGAG	AGAAGCGGAGGAGGC	CCGCTCGCAGGGCCG
TCGGGGCGAGGGAGG	GAAGCGGAGGAGGCG	CGCTCGCAGGGCCGT
CGGGGCGAGGGAGGA	AAGCGGAGGAGGCGG	GCTCGCAGGGCCGTG
GGGGCGAGGGAGGAG	AGCGGAGGAGGCGGC	CTCGCAGGGCCGTGC
GGGCGAGGGAGGAGG	GCGGAGGAGGCGGCT	TCGCAGGGCCGTGCA
GGCGAGGGAGGAGGA	CGGAGGAGGCGGCTC	CGCAGGGCCGTGCAC
GCGAGGGAGGAGGAA	GGAGGAGGCGGCTCC	GCAGGGCCGTGCACC
CGAGGGAGGAGGAAG	GAGGAGGCGGCTCCC	CAGGGCCGTGCACCT
GAGGGAGGAGGAAGA	AGGAGGCGGCTCCCG	AGGGCCGTGCACCTG
AGGGAGGAGGAAGAA	GGAGGCGGCTCCCGC	GGGCCGTGCACCTGC
GGGAGGAGGAAGAAG	GAGGCGGCTCCCGCT	GGCCGTGCACCTGCC
GGAGGAGGAAGAAGC	AGGCGGCTCCCGCTC	GCCGTGCACCTGCCC
GAGGAGGAAGAAGCG	GGCGGCTCCCGCTCG	CCGTGCACCTGCCCG
AGGAGGAAGAAGCGG	GCGGCTCCCGCTCGC	CGTGCACCTGCCCGC
GGAGGAAGAAGCGGA	CGGCTCCCGCTCGCA	GTGCACCTGCCCGCC
GAGGAAGAAGCGGAG	GGCTCCCGCTCGCAG	TGCACCTGCCCGCCC
AGGAAGAAGCGGAGG	GCTCCCGCTCGCAGG	GCACCTGCCCGCCCG
GGAAGAAGCGGAGGA	CTCCCGCTCGCAGGG	CACCTGCCCGCCCGC
GAAGAAGCGGAGGAG	TCCCGCTCGCAGGGC	ACCTGCCCGCCCGCC

CCTGCCCGCCCGCCC
CTGCCCGCCCGCCCG
TGCCCGCCCGCCCGC
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	CGCCCTGCACACCCG	GCCGCGGTGGCCGCA	GGAGCCGGGCTGCGG
	GCCCTGCACACCCGA	CCGCGGTGGCCGCAG	GAGCCGGGCTGCGGC
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	CCTGCACACCCGAGC	GCGGTGGCCGCAGTG	GCCGGGCTGCGGCTG
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	CTGCGGGCCCCCGCC	CCCGCATGCCATGCG	TGCGCCCGGCTGGAG
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CTGCGGCCAGGGGCT CGCTGGTCATGGGCG AGCCCGGAGCAGGTT
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	AGACAATGGCGATGA	CCATGAACATGTTGG	ATGAAGGAGCTGGCC
	GACAATGGCGATGAC	CATGAACATGTTGGG	TGAAGGAGCTGGCCG
5	ACAATGGCGATGACC	ATGAACATGTTGGGC	GAAGGAGCTGGCCGT
	CAATGGCGATGACCA	TGAACATGTTGGGCG	AAGGAGCTGGCCGTG
	AATGGCGATGACCAC	GAACATGTTGGGCGG	AGGAGCTGGCCGTGT
	ATGGCGATGACCACT	AACATGTTGGGCGGG	GGAGCTGGCCGTGTT
	TGGCGATGACCACTC	ACATGTTGGGCGGGG	GAGCTGGCCGTGTTC
10	GGCGATGACCACTCA	CATGTTGGGCGGGGG	AGCTGGCCGTGTTCC
	GCGATGACCACTCAG	ATGTTGGGCGGGGGA	GCTGGCCGTGTTCCG
	CGATGACCACTCAGA	TGTTGGGCGGGGGAG	CTGGCCGTGTTCCGG
	GATGACCACTCAGAA	GTTGGGCGGGGGAGG	TGGCCGTGTTCCGGG
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	ACCACTCAGAAGGAG	GGCGGGGGAGGCAGT	CGTGTTCCGGGAGAA
	CCACTCAGAAGGAGG	GCGGGGGAGGCAGTG	GTGTTCCGGGAGAAG
	CACTCAGAAGGAGGC	CGGGGGAGGCAGTGC	TGTTCCGGGAGAAGG
20	ACTCAGAAGGAGGCC	GGGGGAGGCAGTGCT	GTTCCGGGAGAAGGT
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	TCAGAAGGAGGCCTG	GGGAGGCAGTGCTGG	TCCGGGAGAAGGTCA
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	CCTGGTGGAGAACCA	CTGGCCGGAAGCCCC	GTCACTGAGCAGCAC
	CTGGTGGAGAACCAC	TGGCCGGAAGCCCCT	TCACTGAGCAGCACC
35	TGGTGGAGAACCACG	GGCCGGAAGCCCCTC	CACTGAGCAGCACCG
	GGTGGAGAACCACGT	GCCGGAAGCCCCTCA	ACTGAGCAGCACCGG
	GTGGAGAACCACGTG	CCGGAAGCCCCTCAA	CTGAGCAGCACCGGC
	TGGAGAACCACGTGG	CGGAAGCCCCTCAAG	TGAGCAGCACCGGCA
	GGAGAACCACGTGGA	GGAAGCCCCTCAAGT	GAGCAGCACCGGCAG
40	GAGAACCACGTGGAC	GAAGCCCCTCAAGTC	AGCAGCACCGGCAGA
	AGAACCACGTGGACA	AAGCCCCTCAAGTCG	GCAGCACCGGCAGAT
	GAACCACGTGGACAG	AGCCCCTCAAGTCGG	CAGCACCGGCAGATG
	AACCACGTGGACAGC	GCCCCTCAAGTCGGG	AGCACCGGCAGATGG
	ACCACGTGGACAGCA	CCCCTCAAGTCGGGT	GCACCGGCAGATGGG
45	CCACGTGGACAGCAC	CCCTCAAGTCGGGTA	CACCGGCAGATGGGC
	CACGTGGACAGCACC	CCTCAAGTCGGGTAT	ACCGGCAGATGGGCA
	ACGTGGACAGCACCA	CTCAAGTCGGGTATG	CCGGCAGATGGGCAA
	CGTGGACAGCACCAT	TCAAGTCGGGTATGA	CGGCAGATGGGCAAG
	GTGGACAGCACCATG	CAAGTCGGGTATGAA	GGCAGATGGGCAAGG
50	TGGACAGCACCATGA	AAGTCGGGTATGAAG	GCAGATGGGCAAGGG
	GGACAGCACCATGAA	AGTCGGGTATGAAGG	CAGATGGGCAAGGGT
	GACAGCACCATGAAC	GTCGGGTATGAAGGA	AGATGGGCAAGGGTG
	ACAGCACCATGAACA	TCGGGTATGAAGGAG	GATGGGCAAGGGTGG
	CAGCACCATGAACAT	CGGGTATGAAGGAGC	ATGGGCAAGGGTGGC
55	AGCACCATGAACATG	GGGTATGAAGGAGCT	TGGGCAAGGGTGGCA
	GCACCATGAACATGT	GGTATGAAGGAGCTG	GGGCAAGGGTGGCAA

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GCAAGGGTGGCAAGC
CAAGGGTGGCAAGCA
AAGGGTGGCAAGCAT
AGGGTGGCAAGCATC
GGGTGGCAAGCATCA
GGTGGCAAGCATCAC
GTGGCAAGCATCACC
TGGCAAGCATCACCT
GGCAAGCATCACCTT
GCAAGCATCACCTTG
CAAGCATCACCTTGG
AAGCATCACCTTGGC
AGCATCACCTTGGCC
GCATCACCTTGGCCT
CATCACCTTGGCCTG
ATCACCTTGGCCTGG
TCACCTTGGCCTGGA
CACCTTGGCCTGGAG
ACCTTGGCCTGGAGG
CCTTGGCCTGGAGGA
CTTGGCCTGGAGGAG
TTGGCCTGGAGGAGC
TGGCCTGGAGGAGCC
GGCCTGGAGGAGCCC
GCCTGGAGGAGCCCA
CCTGGAGGAGCCCAA
CTGGAGGAGCCCAAG
TGGAGGAGCCCAAGA
GGAGGAGCCCAAGAA
GAGGAGCCCAAGAAG
AGGAGCCCAAGAAGC
GGAGCCCAAGAAGCT
GAGCCCAAGAAGCTG
AGCCCAAGAAGCTGC
GCCCAAGAAGCTGCG
CCCAAGAAGCTGCGA
CCAAGAAGCTGCGAC
CAAGAAGCTGCGACC
AAGAAGCTGCGACCA
AGAAGCTGCGACCAC
GAAGCTGCGACCACC
AAGCTGCGACCACCC
AGCTGCGACCACCCC
GCTGCGACCACCCCC
CTGCGACCACCCCCT
TGCGACCACCCCCTG
GCGACCACCCCCTGC
CGACCACCCCCTGCC
GACCACCCCCTGCCA
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CCACCCCCTGCCAGG
CACCCCCTGCCAGGA
ACCCCCTGCCAGGAC
CCCCCTGCCAGGACT
CCCCTGCCAGGACTC

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CCTGCCAGGACTCCC
CTGCCAGGACTCCCT
TGCCAGGACTCCCTG
GCCAGGACTCCCTGC
CCAGGACTCCCTGCC
CAGGACTCCCTGCCA
AGGACTCCCTGCCAA
GGACTCCCTGCCAAC
GACTCCCTGCCAACA
ACTCCCTGCCAACAG
CTCCCTGCCAACAGG
TCCCTGCCAACAGGA
CCCTGCCAACAGGAA
CCTGCCAACAGGAAC
CTGCCAACAGGAACT
TGCCAACAGGAACTG
GCCAACAGGAACTGG
CCAACAGGAACTGGA
CAACAGGAACTGGAC
AACAGGAACTGGACC
ACAGGAACTGGACCA
CAGGAACTGGACCAG
AGGAACTGGACCAGG
GGAACTGGACCAGGT
GAACTGGACCAGGTC
AACTGGACCAGGTCC
ACTGGACCAGGTCCT
CTGGACCAGGTCCTG
TGGACCAGGTCCTGG
GGACCAGGTCCTGGA
GACCAGGTCCTGGAG
ACCAGGTCCTGGAGC
CCAGGTCCTGGAGCG
CAGGTCCTGGAGCGG
AGGTCCTGGAGCGGA
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TCCTGGAGCGGATCT
CCTGGAGCGGATCTC
CTGGAGCGGATCTCC
TGGAGCGGATCTCCA
GGAGCGGATCTCCAC
GAGCGGATCTCCACC
AGCGGATCTCCACCA
GCGGATCTCCACCAT
CGGATCTCCACCATG
GGATCTCCACCATGC
GATCTCCACCATGCG
ATCTCCACCATGCGC
TCTCCACCATGCGCC
CTCCACCATGCGCCT
TCCACCATGCGCCTT
CCACCATGCGCCTTC
CACCATGCGCCTTCC
ACCATGCGCCTTCCG

CCATGCGCCTTCCGG
CATGCGCCTTCCGGA
ATGCGCCTTCCGGAT
TGCGCCTTCCGGATG
GCGCCTTCCGGATGA
CGCCTTCCGGATGAG
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CGGGGCCCTCTGGAG
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GGGCCCTCTGGAGCA
GGCCCTCTGGAGCAC
GCCCTCTGGAGCACC
CCCTCTGGAGCACCT
CCTCTGGAGCACCTC
CTCTGGAGCACCTCT
TCTGGAGCACCTCTA
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TGGAGCACCTCTACT
GGAGCACCTCTACTC
GAGCACCTCTACTCC
AGCACCTCTACTCCC
GCACCTCTACTCCCT
CACCTCTACTCCCTG
ACCTCTACTCCCTGC
CCTCTACTCCCTGCA
CTCTACTCCCTGCAC
TCTACTCCCTGCACA
CTACTCCCTGCACAT
TACTCCCTGCACATC
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CTCCCTGCACATCCC
TCCCTGCACATCCCC
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CCTGCACATCCCCAA
CTGCACATCCCCAAC
TGCACATCCCCAACT
GCACATCCCCAACTG
CACATCCCCAACTGT
ACATCCCCAACTGTG
CATCCCCAACTGTGA
ATCCCCAACTGTGAC
TCCCCAACTGTGACA
CCCCAACTGTGACAA

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CCAACTGTGACAAGC
CAACTGTGACAAGCA
AACTGTGACAAGCAT
ACTGTGACAAGCATG
CTGTGACAAGCATGG
TGTGACAAGCATGGC
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TGACAAGCATGGCCT
GACAAGCATGGCCTG
ACAAGCATGGCCTGT
CAAGCATGGCCTGTA
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AGCATGGCCTGTACA
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GCCCCCACCATCCGG
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CCATCCGGGGGGACC
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TGCGGGGTGCACACC
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GCACACCCAGCGGAT
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GACCGCAGCCAGCCG
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CGCAGCCAGCCGGTG
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CAGCCGGTGCCTGGC
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CCCTCTCCAAACACC
CCTCTCCAAACACCG
CTCTCCAAACACCGG
TCTCCAAACACCGGC
CTCCAAACACCGGCA
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GCTGGAGGATTTTCC
CTGGAGGATTTTCCA
TGGAGGATTTTCCAG
GGAGGATTTTCCAGT
GAGGATTTTCCAGTT
AGGATTTTCCAGTTC
GGATTTTCCAGTTCT
GATTTTCCAGTTCTG
ATTTTCCAGTTCTGA
TTTTCCAGTTCTGAC
TTTCCAGTTCTGACA
TTCCAGTTCTGACAC
TCCAGTTCTGACACA
CCAGTTCTGACACAC
CAGTTCTGACACACG
AGTTCTGACACACGT
GTTCTGACACACGTA
TTCTGACACACGTAT
TCTGACACACGTATT
CTGACACACGTATTT
TGACACACGTATTTA
GACACACGTATTTAT
ACACACGTATTTATA
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ACGTATTTATATTTG
CGTATTTATATTTGG
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ATATTTGGAAAGAGA
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ATTTGGAAAGAGACC
TTTGGAAAGAGACCA
TTGGAAAGAGACCAG
TGGAAAGAGACCAGC
GGAAAGAGACCAGCA
GAAAGAGACCAGCAC
AAAGAGACCAGCACC
AAGAGACCAGCACCG
AGAGACCAGCACCGA
GAGACCAGCACCGAG
AGACCAGCACCGAGC

	GACCAGCACCGAGCT	CACCTGCTCCTTCTT	GGGTACAGGTTTGGG
	ACCAGCACCGAGCTC	ACCTGCTCCTTCTTG	GGTACAGGTTTGGGG
	CCAGCACCGAGCTCG	CCTGCTCCTTCTTGC	GTACAGGTTTGGGGA
	CAGCACCGAGCTCGG	CTGCTCCTTCTTGCT	TACAGGTTTGGGGAG
5	AGCACCGAGCTCGGC	TGCTCCTTCTTGCTT	ACAGGTTTGGGGAGG
	GCACCGAGCTCGGCA	GCTCCTTCTTGCTTT	CAGGTTTGGGGAGGG
	CACCGAGCTCGGCAC	CTCCTTCTTGCTTTC	AGGTTTGGGGAGGGG
	ACCGAGCTCGGCACC	TCCTTCTTGCTTTCC	GGTTTGGGGAGGGGG
	CCGAGCTCGGCACCT	CCTTCTTGCTTTCCC	GTTTGGGGAGGGGGA
10	CGAGCTCGGCACCTC	CTTCTTGCTTTCCCC	TTTGGGGAGGGGGAA
	GAGCTCGGCACCTCC	TTCTTGCTTTCCCCG	TTGGGGAGGGGGAAG
	AGCTCGGCACCTCCC	TCTTGCTTTCCCCGC	TGGGGAGGGGGAAGA
	GCTCGGCACCTCCCC	CTTGCTTTCCCCGGG	GGGGAGGGGGAAGAG
	CTCGGCACCTCCCCG	TTGCTTTCCCCGGGG	GGGAGGGGGAAGAGA
15	TCGGCACCTCCCCGG	TGCTTTCCCCGGGGG	GGAGGGGGAAGAGAA
	CGGCACCTCCCCGGC	GCTTTCCCCGGGGGA	GAGGGGGAAGAGAAA
	GGCACCTCCCCGGCC	CTTTCCCCGGGGGAG	AGGGGGAAGAGAAAT
	GCACCTCCCCGGCCT	TTTCCCCGGGGGAGG	GGGGGAAGAGAAATT
	CACCTCCCCGGCCTC	TTCCCCGGGGGAGGA	GGGGAAGAGAAATTT
20	ACCTCCCCGGCCTCT	TCCCCGGGGGAGGAA	GGGAAGAGAAATTTT
	CCTCCCCGGCCTCTC	CCCCGGGGGAGGAAG	GGAAGAGAAATTTTT
	CTCCCCGGCCTCTCT	CCCGGGGGAGGAAGG	GAAGAGAAATTTTTA
	TCCCCGGCCTCTCTC	CCGGGGGAGGAAGGG	AAGAGAAATTTTTAT
	CCCCGGCCTCTCTCT	CGGGGGAGGAAGGGG	AGAGAAATTTTTATT
25	CCCGGCCTCTCTCTT	GGGGGAGGAAGGGGG	GAGAAATTTTTATTT
	CCGGCCTCTCTCTTC	GGGGAGGAAGGGGGT	AGAAATTTTTATTTT
	CGGCCTCTCTCTTCC	GGGAGGAAGGGGGTT	GAAATTTTTATTTTT
	GGCCTCTCTCTTCCC	GGAGGAAGGGGGTTG	AAATTTTTATTTTTG
	GCCTCTCTCTTCCCA	GAGGAAGGGGGTTGT	AATTTTTATTTTTGA
30	CCTCTCTCTTCCCAG	AGGAAGGGGGTTGTG	ATTTTTATTTTTGAA
	CTCTCTCTTCCCAGC	GGAAGGGGGTTGTGG	TTTTTATTTTTGAAC
	TCTCTCTTCCCAGCT	GAAGGGGGTTGTGGT	TTTTATTTTTGAACC
	CTCTCTTCCCAGCTG	AAGGGGGTTGTGGTC	TTTATTTTTGAACCC
	TCTCTTCCCAGCTGC	AGGGGGTTGTGGTCG	TTATTTTTGAACCCC
35	CTCTTCCCAGCTGCA	GGGGGTTGTGGTCGG	TATTTTTGAACCCCT
	TCTTCCCAGCTGCAG	GGGGTTGTGGTCGGG	ATTTTTGAACCCCTG
	CTTCCCAGCTGCAGA	GGGTTGTGGTCGGGG	TTTTTGAACCCCTGT
	TTCCCAGCTGCAGAT	GGTTGTGGTCGGGGA	TTTTGAACCCCTGTG
	TCCCAGCTGCAGATG	GTTGTGGTCGGGGAG	TTTGAACCCCTGTGT
40	CCCAGCTGCAGATGC	TTGTGGTCGGGGAGC	TTGAACCCCTGTGTC
	CCAGCTGCAGATGCC	TGTGGTCGGGGAGCT	TGAACCCCTGTGTCC
	CAGCTGCAGATGCCA	GTGGTCGGGGAGCTG	GAACCCCTGTGTCCC
	AGCTGCAGATGCCAC	TGGTCGGGGAGCTGG	AACCCCTGTGTCCCT
	GCTGCAGATGCCACA	GGTCGGGGAGCTGGG	ACCCCTGTGTCCCTT
45	CTGCAGATGCCACAC	GTCGGGGAGCTGGGG	CCCCTGTGTCCCTTT
	TGCAGATGCCACACC	TCGGGGAGCTGGGGT	CCCTGTGTCCCTTTT
	GCAGATGCCACACCT	CGGGGAGCTGGGGTA	CCTGTGTCCCTTTTG
	CAGATGCCACACCTG	GGGGAGCTGGGGTAC	CTGTGTCCCTTTTGC
	AGATGCCACACCTGC	GGGAGCTGGGGTACA	TGTGTCCCTTTTGCA
50	GATGCCACACCTGCT	GGAGCTGGGGTACAG	GTGTCCCTTTTGCAT
	ATGCCACACCTGCTC	GAGCTGGGGTACAGG	TGTCCCTTTTGCATA
	TGCCACACCTGCTCC	AGCTGGGGTACAGGT	GTCCCTTTTGCATAA
	GCCACACCTGCTCCT	GCTGGGGTACAGGTT	TCCCTTTTGCATAAG
	CCACACCTGCTCCTT	CTGGGGTACAGGTTT	CCCTTTTGCATAAGA
55	CACACCTGCTCCTTC	TGGGGTACAGGTTTG	CCTTTTGCATAAGAT
	ACACCTGCTCCTTCT	GGGGTACAGGTTTGG	CTTTTGCATAAGATT

TTTTGCATAAGATTA
TTTGCATAAGATTAA
TTGCATAAGATTAAA
TGCATAAGATTAAAG
GCATAAGATTAAAGG
CATAAGATTAAAGGA
ATAAGATTAAAGGAA
TAAGATTAAAGGAAG
AAGATTAAAGGAAGG
AGATTAAAGGAAGGA
GATTAAAGGAAGGAA
ATTAAAGGAAGGAAA
TTAAAGGAAGGAAAA
TAAAGGAAGGAAAAG
AAAGGAAGGAAAAGT

EXAMPLE 7

ANTISENSE OLIGONUCLEOTIDES OF IGFBP3

Antisense oligonucleotides to IGFBP3 may be selected from molecules capable of interacting with one or more of the following sense oligonucleotides:

CTCAGCGCCCAGCCG
TCAGCGCCCAGCCGC
CAGCGCCCAGCCGCT
AGCGCCCAGCCGCTT
GCGCCCAGCCGCTTC
CGCCCAGCCGCTTCC
GCCCAGCCGCTTCCT
CCCAGCCGCTTCCTG
CCAGCCGCTTCCTGC
CAGCCGCTTCCTGCC
AGCCGCTTCCTGCCT
GCCGCTTCCTGCCTG
CCGCTTCCTGCCTGG
CGCTTCCTGCCTGGA
GCTTCCTGCCTGGAT
CTTCCTGCCTGGATT
TTCCTGCCTGGATTC
TCCTGCCTGGATTCC
CCTGCCTGGATTCCA
CTGCCTGGATTCCAC
TGCCTGGATTCCACA
GCCTGGATTCCACAG
CCTGGATTCCACAGC
CTGGATTCCACAGCT
TGGATTCCACAGCTT
GGATTCCACAGCTTC
GATTCCACAGCTTCG
ATTCCACAGCTTCGC
TTCCACAGCTTCGCG
TCCACAGCTTCGCGC

CCACAGCTTCGCGCC
CACAGCTTCGCGCCG
ACAGCTTCGCGCCGT
CAGCTTCGCGCCGTG
AGCTTCGCGCCGTGT
GCTTCGCGCCGTGTA
CTTCGCGCCGTGTAC
TTCGCGCCGTGTACT
TCGCGCCGTGTACTG
CGCGCCGTGTACTGT
GCGCCGTGTACTGTC
CGCCGTGTACTGTCG
GCCGTGTACTGTCGC
CCGTGTACTGTCGCC
CGTGTACTGTCGCCC
GTGTACTGTCGCCCC
TGTACTGTCGCCCCA
GTACTGTCGCCCCAT
TACTGTCGCCCCATC
ACTGTCGCCCCATCC
CTGTCGCCCCATCCC
TGTCGCCCCATCCCT
GTCGCCCCATCCCTG
TCGCCCCATCCCTGC
CGCCCCATCCCTGCG
GCCCCATCCCTGCGC
CCCCATCCCTGCGCG
CCCATCCCTGCGCGC
CCATCCCTGCGCGCC
CATCCCTGCGCGCCC

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TCCCTGCGCGCCCAG
CCCTGCGCGCCCAGC
CCTGCGCGCCCAGCC
CTGCGCGCCCAGCCT
TGCGCGCCCAGCCTG
GCGCGCCCAGCCTGC
CGCGCCCAGCCTGCC
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CGCCCAGCCTGCCAA
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CCCAGCCTGCCAAGC
CCAGCCTGCCAAGCA
CAGCCTGCCAAGCAG
AGCCTGCCAAGCAGC
GCCTGCCAAGCAGCG
CCTGCCAAGCAGCGT
CTGCCAAGCAGCGTG
TGCCAAGCAGCGTGC
GCCAAGCAGCGTGCC
CCAAGCAGCGTGCCC
CAAGCAGCGTGCCCC
AAGCAGCGTGCCCCG
AGCAGCGTGCCCCGG
GCAGCGTGCCCCGGT
CAGCGTGCCCCGGTT
AGCGTGCCCCGGTTG
GCGTGCCCCGGTTGC
CGTGCCCCGGTTGCA
GTGCCCCGGTTGCAG

TGCCCCGGTTGCAGG
GCCCCGGTTGCAGGC
CCCCGGTTGCAGGCG
CCCGGTTGCAGGCGT
CCGGTTGCAGGCGTC
CGGTTGCAGGCGTCA
GGTTGCAGGCGTCAT
GTTGCAGGCGTCATG
TTGCAGGCGTCATGC
TGCAGGCGTCATGCA
GCAGGCGTCATGCAG
CAGGCGTCATGCAGC
AGGCGTCATGCAGCG
GGCGTCATGCAGCGG
GCGTCATGCAGCGGG
CGTCATGCAGCGGGC
GTCATGCAGCGGGCG
TCATGCAGCGGGCGC
CATGCAGCGGGCGCG
ATGCAGCGGGCGCGA
TGCAGCGGGCGCGAC
GCAGCGGGCGCGACC
CAGCGGGCGCGACCC
AGCGGGCGCGACCCA
GCGGGCGCGACCCAC
CGGGCGCGACCCACG
GGGCGCGACCCACGC
GGCGCGACCCACGCT
GCGCGACCCACGCTC
CGCGACCCACGCTCT
GCGACCCACGCTCTG
CGACCCACGCTCTGG
GACCCACGCTCTGGG
ACCCACGCTCTGGGC
CCCACGCTCTGGGCC
CCACGCTCTGGGCCG
CACGCTCTGGGCCGC
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CGCTCTGGGCCGCTG
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GGGCCGCTGCGCTGA
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GCGCTGACTCTGCTG
CGCTGACTCTGCTGG
GCTGACTCTGCTGGT
CTGACTCTGCTGGTG

TGACTCTGCTGGTGC
GACTCTGCTGGTGCT
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	TATCAGAACAGGAAG	CACATAGAGATTCAC	ATAATTCACACATAT
	ATCAGAACAGGAAGG	ACATAGAGATTCACC	TAATTCACACATATA
	TCAGAACAGGAAGGA	CATAGAGATTCACCC	AATTCACACATATAT
15	CAGAACAGGAAGGAG	ATAGAGATTCACCCA	ATTCACACATATATG
	AGAACAGGAAGGAGG	TAGAGATTCACCCAT	TTCACACATATATGC
	GAACAGGAAGGAGGA	AGAGATTCACCCATG	TCACACATATATGCA
	AACAGGAAGGAGGAA	GAGATTCACCCATGT	CACACATATATGCAG
	ACAGGAAGGAGGAAT	AGATTCACCCATGTT	ACACATATATGCAGA
20	CAGGAAGGAGGAATG	GATTCACCCATGTTT	CACATATATGCAGAG
	AGGAAGGAGGAATGG	ATTCACCCATGTTTG	ACATATATGCAGAGA
	GGAAGGAGGAATGGC	TTCACCCATGTTTGT	CATATATGCAGAGAA
	GAAGGAGGAATGGCT	TCACCCATGTTTGTT	ATATATGCAGAGAAG
	AAGGAGGAATGGCTT	CACCCATGTTTGTTG	TATATGCAGAGAAGA
25	AGGAGGAATGGCTTG	ACCCATGTTTGTTGA	ATATGCAGAGAAGAT
	GGAGGAATGGCTTGC	CCCATGTTTGTTGAA	TATGCAGAGAAGATA
	GAGGAATGGCTTGCT	CCATGTTTGTTGAAC	ATGCAGAGAAGATAT
	AGGAATGGCTTGCTG	CATGTTTGTTGAACT	TGCAGAGAAGATATG
	GGAATGGCTTGCTGG	ATGTTTGTTGAACTT	GCAGAGAAGATATGT
30	GAATGGCTTGCTGGG	TGTTTGTTGAACTTA	CAGAGAAGATATGTT
	AATGGCTTGCTGGGG	GTTTGTTGAACTTAG	AGAGAAGATATGTTC
	ATGGCTTGCTGGGGA	TTTGTTGAACTTAGA	GAGAAGATATGTTCT
	TGGCTTGCTGGGGAG	TTGTTGAACTTAGAG	AGAAGATATGTTCTT
	GGCTTGCTGGGGAGC	TGTTGAACTTAGAGT	GAAGATATGTTCTTG
35	GCTTGCTGGGGAGCC	GTTGAACTTAGAGTC	AAGATATGTTCTTGT
	CTTGCTGGGGAGCCC	TTGAACTTAGAGTCA	AGATATGTTCTTGTT
	TTGCTGGGGAGCCCA	TGAACTTAGAGTCAT	GATATGTTCTTGTTA
	TGCTGGGGAGCCCAT	GAACTTAGAGTCATT	ATATGTTCTTGTTAA
	GCTGGGGAGCCCATC	AACTTAGAGTCATTC	TATGTTCTTGTTAAC
40	CTGGGGAGCCCATCC	ACTTAGAGTCATTCT	ATGTTCTTGTTAACA
	TGGGGAGCCCATCCA	CTTAGAGTCATTCTC	TGTTCTTGTTAACAT
	GGGGAGCCCATCCAG	TTAGAGTCATTCTCA	GTTCTTGTTAACATT
	GGGAGCCCATCCAGG	TAGAGTCATTCTCAT	TTCTTGTTAACATTG
	GGAGCCCATCCAGGA	AGAGTCATTCTCATG	TCTTGTTAACATTGT
45	GAGCCCATCCAGGAC	GAGTCATTCTCATGC	CTTGTTAACATTGTA
	AGCCCATCCAGGACA	AGTCATTCTCATGCT	TTGTTAACATTGTAT
	GCCCATCCAGGACAC	GTCATTCTCATGCTT	TGTTAACATTGTATA
	CCCATCCAGGACACT	TCATTCTCATGCTTT	GTTAACATTGTATAC
	CCATCCAGGACACTG	CATTCTCATGCTTTT	TTAACATTGTATACA
50	CATCCAGGACACTGG	ATTCTCATGCTTTTC	TAACATTGTATACAA
	ATCCAGGACACTGGG	TTCTCATGCTTTTCT	AACATTGTATACAAC
	TCCAGGACACTGGGA	TCTCATGCTTTTCTT	ACATTGTATACAACA
	CCAGGACACTGGGAG	CTCATGCTTTTCTTT	CATTGTATACAACAT
	CAGGACACTGGGAGC	TCATGCTTTTCTTTA	ATTGTATACAACATA
55	AGGACACTGGGAGCA	CATGCTTTTCTTTAT	TTGTATACAACATAG
	GGACACTGGGAGCAC	ATGCTTTTCTTTATA	TGTATACAACATAGC

	GTATACAACATAGCC	TGTTAGAGATGCTAT	CCAGAGACTGGGCTG
	TATACAACATAGCCC	GTTAGAGATGCTATA	CAGAGACTGGGCTGC
	ATACAACATAGCCCC	TTAGAGATGCTATAT	AGAGACTGGGCTGCT
	TACAACATAGCCCCA	TAGAGATGCTATATG	GAGACTGGGCTGCTC
5	ACAACATAGCCCCAA	AGAGATGCTATATGA	AGACTGGGCTGCTCT
	CAACATAGCCCCAAA	GAGATGCTATATGAT	GACTGGGCTGCTCTC
	AACATAGCCCCAAAT	AGATGCTATATGATA	ACTGGGCTGCTCTCC
	ACATAGCCCCAAATA	GATGCTATATGATAC	CTGGGCTGCTCTCCC
	CATAGCCCCAAATAT	ATGCTATATGATACA	TGGGCTGCTCTCCCG
10	ATAGCCCCAAATATA	TGCTATATGATACAA	GGGCTGCTCTCCCGG
	TAGCCCCAAATATAG	GCTATATGATACAAC	GGCTGCTCTCCCGGA
	AGCCCCAAATATAGT	CTATATGATACAACT	GCTGCTCTCCCGGAG
	GCCCCAAATATAGTA	TATATGATACAACTG	CTGCTCTCCCGGAGG
	CCCCAAATATAGTAA	ATATGATACAACTGT	TGCTCTCCCGGAGGC
15	CCCAAATATAGTAAG	TATGATACAACTGTG	GCTCTCCCGGAGGCC
	CCAAATATAGTAAGA	ATGATACAACTGTGG	CTCTCCCGGAGGCCA
	CAAATATAGTAAGAT	TGATACAACTGTGGC	TCTCCCGGAGGCCAA
	AAATATAGTAAGATC	GATACAACTGTGGCC	CTCCCGGAGGCCAAA
	AATATAGTAAGATCT	ATACAACTGTGGCCA	TCCCGGAGGCCAAAC
20	ATATAGTAAGATCTA	TACAACTGTGGCCAT	CCCGGAGGCCAAACC
	TATAGTAAGATCTAT	ACAACTGTGGCCATG	CCGGAGGCCAAACCC
	ATAGTAAGATCTATA	CAACTGTGGCCATGA	CGGAGGCCAAACCCA
	TAGTAAGATCTATAC	AACTGTGGCCATGAC	GGAGGCCAAACCCAA
	AGTAAGATCTATACT	ACTGTGGCCATGACT	GAGGCCAAACCCAAG
25	GTAAGATCTATACTA	CTGTGGCCATGACTG	AGGCCAAACCCAAGA
	TAAGATCTATACTAG	TGTGGCCATGACTGA	GGCCAAACCCAAGAA
	AAGATCTATACTAGA	GTGGCCATGACTGAG	GCCAAACCCAAGAAG
	AGATCTATACTAGAT	TGGCCATGACTGAGG	CCAAACCCAAGAAGG
	GATCTATACTAGATA	GGCCATGACTGAGGA	CAAACCCAAGAAGGT
30	ATCTATACTAGATAA	GCCATGACTGAGGAA	AAACCCAAGAAGGTC
	TCTATACTAGATAAT	CCATGACTGAGGAAA	AACCCAAGAAGGTCT
	CTATACTAGATAATC	CATGACTGAGGAAAG	ACCCAAGAAGGTCTG
	TATACTAGATAATCC	ATGACTGAGGAAAGG	CCCAAGAAGGTCTGG
	ATACTAGATAATCCT	TGACTGAGGAAAGGA	CCAAGAAGGTCTGGC
35	TACTAGATAATCCTA	GACTGAGGAAAGGAG	CAAGAAGGTCTGGCA
	ACTAGATAATCCTAG	ACTGAGGAAAGGAGC	AAGAAGGTCTGGCAA
	CTAGATAATCCTAGA	CTGAGGAAAGGAGCT	AGAAGGTCTGGCAAA
	TAGATAATCCTAGAT	TGAGGAAAGGAGCTC	GAAGGTCTGGCAAAG
	AGATAATCCTAGATG	GAGGAAAGGAGCTCA	AAGGTCTGGCAAAGT
40	GATAATCCTAGATGA	AGGAAAGGAGCTCAC	AGGTCTGGCAAAGTC
	ATAATCCTAGATGAA	GGAAAGGAGCTCACG	GGTCTGGCAAAGTCA
	TAATCCTAGATGAAA	GAAAGGAGCTCACGC	GTCTGGCAAAGTCAG
	AATCCTAGATGAAAT	AAAGGAGCTCACGCC	TCTGGCAAAGTCAGG
	ATCCTAGATGAAATG	AAGGAGCTCACGCCC	CTGGCAAAGTCAGGC
45	TCCTAGATGAAATGT	AGGAGCTCACGCCCA	TGGCAAAGTCAGGCT
	CCTAGATGAAATGTT	GGAGCTCACGCCCAG	GGCAAAGTCAGGCTC
	CTAGATGAAATGTTA	GAGCTCACGCCCAGA	GCAAAGTCAGGCTCA
	TAGATGAAATGTTAG	AGCTCACGCCCAGAG	CAAAGTCAGGCTCAG
	AGATGAAATGTTAGA	GCTCACGCCCAGAGA	AAAGTCAGGCTCAGG
50	GATGAAATGTTAGAG	CTCACGCCCAGAGAC	AAGTCAGGCTCAGGG
	ATGAAATGTTAGAGA	TCACGCCCAGAGACT	AGTCAGGCTCAGGGA
	TGAAATGTTAGAGAT	CACGCCCAGAGACTG	GTCAGGCTCAGGGAG
	GAAATGTTAGAGATG	ACGCCCAGAGACTGG	TCAGGCTCAGGGAGA
	AAATGTTAGAGATGC	CGCCCAGAGACTGGG	CAGGCTCAGGGAGAC
55	AATGTTAGAGATGCT	GCCCAGAGACTGGGC	AGGCTCAGGGAGACT
	ATGTTAGAGATGCTA	CCCAGAGACTGGGCT	GGCTCAGGGAGACTC

	GCTCAGGGAGACTCT	CTCTCCTTGAAAACA	TATTTTTTTAACTTT
	CTCAGGGAGACTCTG	TCTCCTTGAAAACAG	ATTTTTTTAACTTTT
	TCAGGGAGACTCTGC	CTCCTTGAAAACAGA	TTTTTTTAACTTTTT
	CAGGGAGACTCTGCC	TCCTTGAAAACAGAG	TTTTTTAACTTTTTG
5	AGGGAGACTCTGCCC	CCTTGAAAACAGAGG	TTTTTAACTTTTTGG
	GGGAGACTCTGCCCT	CTTGAAAACAGAGGG	TTTTAACTTTTTGGG
	GGAGACTCTGCCCTG	TTGAAAACAGAGGGG	TTTAACTTTTTGGGG
	GAGACTCTGCCCTGC	TGAAAACAGAGGGGT	TTAACTTTTTGGGGG
	AGACTCTGCCCTGCT	GAAAACAGAGGGGTC	TAACTTTTTGGGGGG
10	GACTCTGCCCTGCTG	AAAACAGAGGGGTCT	AACTTTTTGGGGGGA
	ACTCTGCCCTGCTGC	AAACAGAGGGGTCTC	ACTTTTTGGGGGGAA
	CTCTGCCCTGCTGCA	AACAGAGGGGTCTCA	CTTTTTGGGGGGAAA
	TCTGCCCTGCTGCAG	ACAGAGGGGTCTCAA	TTTTTGGGGGGAAAA
	CTGCCCTGCTGCAGA	CAGAGGGGTCTCAAG	TTTTGGGGGGAAAAG
15	TGCCCTGCTGCAGAC	AGAGGGGTCTCAAGA	TTTGGGGGGAAAAGT
	GCCCTGCTGCAGACC	GAGGGGTCTCAAGAC	TTGGGGGGAAAAGTA
	CCCTGCTGCAGACCT	AGGGGTCTCAAGACA	TGGGGGGAAAAGTAT
	CCTGCTGCAGACCTC	GGGGTCTCAAGACAT	GGGGGGAAAAGTATT
	CTGCTGCAGACCTCG	GGGTCTCAAGACATT	GGGGGAAAAGTATTT
20	TGCTGCAGACCTCGG	GGTCTCAAGACATTC	GGGGAAAAGTATTTT
	GCTGCAGACCTCGGT	GTCTCAAGACATTCT	GGGAAAAGTATTTTT
	CTGCAGACCTCGGTG	TCTCAAGACATTCTG	GGAAAAGTATTTTTG
	TGCAGACCTCGGTGT	CTCAAGACATTCTGC	GAAAAGTATTTTTGA
	GCAGACCTCGGTGTG	TCAAGACATTCTGCC	AAAAGTATTTTTGAG
25	CAGACCTCGGTGTGG	CAAGACATTCTGCCT	AAAGTATTTTTGAGA
	AGACCTCGGTGTGGA	AAGACATTCTGCCTA	AAGTATTTTTGAGAA
	GACCTCGGTGTGGAC	AGACATTCTGCCTAC	AGTATTTTTGAGAAG
	ACCTCGGTGTGGACA	GACATTCTGCCTACC	GTATTTTTGAGAAGT
	CCTCGGTGTGGACAC	ACATTCTGCCTACCT	TATTTTTGAGAAGTT
30	CTCGGTGTGGACACA	CATTCTGCCTACCTA	ATTTTTGAGAAGTTT
	TCGGTGTGGACACAC	ATTCTGCCTACCTAT	TTTTTGAGAAGTTTG
	CGGTGTGGACACACG	TTCTGCCTACCTATT	TTTTGAGAAGTTTGT
	GGTGTGGACACACGC	TCTGCCTACCTATTA	TTTGAGAAGTTTGTC
	GTGTGGACACACGCT	CTGCCTACCTATTAG	TTGAGAAGTTTGTCT
35	TGTGGACACACGCTG	TGCCTACCTATTAGC	TGAGAAGTTTGTCTT
	GTGGACACACGCTGC	GCCTACCTATTAGCT	GAGAAGTTTGTCTTG
	TGGACACACGCTGCA	CCTACCTATTAGCTT	AGAAGTTTGTCTTGC
	GGACACACGCTGCAT	CTACCTATTAGCTTT	GAAGTTTGTCTTGCA
	GACACACGCTGCATA	TACCTATTAGCTTTT	AAGTTTGTCTTGCAA
40	ACACACGCTGCATAG	ACCTATTAGCTTTTC	AGTTTGTCTTGCAAT
	CACACGCTGCATAGA	CCTATTAGCTTTTCT	GTTTGTCTTGCAATG
	ACACGCTGCATAGAG	CTATTAGCTTTTCTT	TTTGTCTTGCAATGT
	CACGCTGCATAGAGC	TATTAGCTTTTCTTT	TTGTCTTGCAATGTA
	ACGCTGCATAGAGCT	ATTAGCTTTTCTTTA	TGTCTTGCAATGTAT
45	CGCTGCATAGAGCTC	TTAGCTTTTCTTTAT	GTCTTGCAATGTATT
	GCTGCATAGAGCTCT	TAGCTTTTCTTTATT	TCTTGCAATGTATTT
	CTGCATAGAGCTCTC	AGCTTTTCTTTATTT	CTTGCAATGTATTTA
	TGCATAGAGCTCTCC	GCTTTTCTTTATTTT	TTGCAATGTATTTAT
	GCATAGAGCTCTCCT	CTTTTCTTTATTTTT	TGCAATGTATTTATA
50	CATAGAGCTCTCCTT	TTTTCTTTATTTTTT	GCAATGTATTTATAA
	ATAGAGCTCTCCTTG	TTTCTTTATTTTTTT	CAATGTATTTATAAA
	TAGAGCTCTCCTTGA	TTCTTTATTTTTTTA	AATGTATTTATAAAT
	AGAGCTCTCCTTGAA	TCTTTATTTTTTTAA	ATGTATTTATAAATA
	GAGCTCTCCTTGAAA	CTTTATTTTTTTAAC	TGTATTTATAAATAG
55	AGCTCTCCTTGAAAA	TTTATTTTTTTAACT	GTATTTATAAATAGT
	GCTCTCCTTGAAAAC	TTATTTTTTTAACTT	TATTTATAAATAGTA

ATTTATAAATAGTAA
TTTATAAATAGTAAA
TTATAAATAGTAAAT
TATAAATAGTAAATA
ATAAATAGTAAATAA
TAAATAGTAAATAAA
AAATAGTAAATAAAG
AATAGTAAATAAAGT
ATAGTAAATAAAGTT
TAGTAAATAAAGTTT
AGTAAATAAAGTTTT
GTAAATAAAGTTTTT
TAAATAAAGTTTTTA
AAATAAAGTTTTTAC
AATAAAGTTTTTACC
ATAAAGTTTTTACCA
TAAAGTTTTTACCAT
AAAGTTTTTACCATT

EXAMPLE 8

ANTISENSE OLIGONUCLEOTIDES OF IGF-I RECEPTOR

Antisense oligonucleotides to IGF-I may be selected from molecules capable of interacting with one or more of the following sense oligonucleotides:

TTTTTTTTTTTTTTG	TCATCCCAAATAAAA	GGCTCCGGAGGAGGG
TTTTTTTTTTTTTGA	CATCCCAAATAAAAG	GCTCCGGAGGAGGGT
TTTTTTTTTTTTGAG	ATCCCAAATAAAAGG	CTCCGGAGGAGGGTC
TTTTTTTTTTTGAGA	TCCCAAATAAAAGGA	TCCGGAGGAGGGTCC
TTTTTTTTTTGAGAA	CCCAAATAAAAGGAA	CCGGAGGAGGGTCCC
TTTTTTTTTGAGAAA	CCAAATAAAAGGAAT	CGGAGGAGGGTCCCC
TTTTTTTTGAGAAAG	CAAATAAAAGGAATG	GGAGGAGGGTCCCCG
TTTTTTTGAGAAAGG	AAATAAAAGGAATGA	GAGGAGGGTCCCCGA
TTTTTTGAGAAAGGG	AATAAAAGGAATGAA	AGGAGGGTCCCCGAC
TTTTTGAGAAAGGGA	ATAAAAGGAATGAAG	GGAGGGTCCCCGACC
TTTTGAGAAAGGGAA	TAAAAGGAATGAAGT	GAGGGTCCCCGACCT
TTTGAGAAAGGGAAT	AAAAGGAATGAAGTC	AGGGTCCCCGACCTC
TTGAGAAAGGGAATT	AAAGGAATGAAGTCT	GGGTCCCCGACCTCG
TGAGAAAGGGAATTT	AAGGAATGAAGTCTG	GGTCCCCGACCTCGC
GAGAAAGGGAATTTC	AGGAATGAAGTCTGG	GTCCCCGACCTCGCT
AGAAAGGGAATTTCA	GGAATGAAGTCTGGC	TCCCCGACCTCGCTG
GAAAGGGAATTTCAT	GAATGAAGTCTGGCT	CCCCGACCTCGCTGT
AAAGGGAATTTCATC	AATGAAGTCTGGCTC	CCCGACCTCGCTGTG
AAGGGAATTTCATCC	ATGAAGTCTGGCTCC	CCGACCTCGCTGTGG
AGGGAATTTCATCCC	TGAAGTCTGGCTCCG	CGACCTCGCTGTGGG
GGGAATTTCATCCCA	GAAGTCTGGCTCCGG	GACCTCGCTGTGGGG
GGAATTTCATCCCAA	AAGTCTGGCTCCGGA	ACCTCGCTGTGGGGG
GAATTTCATCCCAAA	AGTCTGGCTCCGGAG	CCTCGCTGTGGGGGC
AATTTCATCCCAAAT	GTCTGGCTCCGGAGG	CTCGCTGTGGGGGCT
ATTTCATCCCAAATA	TCTGGCTCCGGAGGA	TCGCTGTGGGGGCTC
TTTCATCCCAAATAA	CTGGCTCCGGAGGAG	CGCTGTGGGGGCTCC
TTCATCCCAAATAAA	TGGCTCCGGAGGAGG	GCTGTGGGGGCTCCT

CTGTGGGGGCTCCTG AATCTGCGGGCCAGG AGAACTGCACGGTGA
TGTGGGGGCTCCTGT ATCTGCGGGCCAGGC GAACTGCACGGTGAT
GTGGGGGCTCCTGTT TCTGCGGGCCAGGCA AACTGCACGGTGATC
TGGGGGCTCCTGTTT CTGCGGGCCAGGCAT ACTGCACGGTGATCG
GGGGGCTCCTGTTTC TGCGGGCCAGGCATC CTGCACGGTGATCGA
GGGGCTCCTGTTTCT GCGGGCCAGGCATCG TGCACGGTGATCGAG
GGGCTCCTGTTTCTC CGGGCCAGGCATCGA GCACGGTGATCGAGG
GGCTCCTGTTTCTCT GGGCCAGGCATCGAC CACGGTGATCGAGGG
GCTCCTGTTTCTCTC GGCCAGGCATCGACA ACGGTGATCGAGGGC
CTCCTGTTTCTCTCC GCCAGGCATCGACAT CGGTGATCGAGGGCT
TCCTGTTTCTCTCCG CCAGGCATCGACATC GGTGATCGAGGGCTA
CCTGTTTCTCTCCGC CAGGCATCGACATCC GTGATCGAGGGCTAC
CTGTTTCTCTCCGCC AGGCATCGACATCCG TGATCGAGGGCTACC
TGTTTCTCTCCGCCG GGCATCGACATCCGC GATCGAGGGCTACCT
GTTTCTCTCCGCCGC GCATCGACATCCGCA ATCGAGGGCTACCTC
TTTCTCTCCGCCGCG CATCGACATCCGCAA TCGAGGGCTACCTCC
TTCTCTCCGCCGCGC ATCGACATCCGCAAC CGAGGGCTACCTCCA
TCTCTCCGCCGCGCT TCGACATCCGCAACG GAGGGCTACCTCCAC
CTCTCCGCCGCGCTC CGACATCCGCAACGA AGGGCTACCTCCACA
TCTCCGCCGCGCTCT GACATCCGCAACGAC GGGCTACCTCCACAT
CTCCGCCGCGCTCTC ACATCCGCAACGACT GGCTACCTCCACATC
TCCGCCGCGCTCTCG CATCCGCAACGACTA GCTACCTCCACATCC
CCGCCGCGCTCTCGC ATCCGCAACGACTAT CTACCTCCACATCCT
CGCCGCGCTCTCGCT TCCGCAACGACTATC TACCTCCACATCCTG
GCCGCGCTCTCGCTC CCGCAACGACTATCA ACCTCCACATCCTGC
CCGCGCTCTCGCTCT CGCAACGACTATCAG CCTCCACATCCTGCT
CGCGCTCTCGCTCTG GCAACGACTATCAGC CTCCACATCCTGCTC
GCGCTCTCGCTCTGG CAACGACTATCAGCA TCCACATCCTGCTCA
CGCTCTCGCTCTGGC AACGACTATCAGCAG CCACATCCTGCTCAT
GCTCTCGCTCTGGCC ACGACTATCAGCAGC CACATCCTGCTCATC
CTCTCGCTCTGGCCG CGACTATCAGCAGCT ACATCCTGCTCATCT
TCTCGCTCTGGCCGA GACTATCAGCAGCTG CATCCTGCTCATCTC
CTCGCTCTGGCCGAC ACTATCAGCAGCTGA ATCCTGCTCATCTCC
TCGCTCTGGCCGACG CTATCAGCAGCTGAA TCCTGCTCATCTCCA
CGCTCTGGCCGACGA TATCAGCAGCTGAAG CCTGCTCATCTCCAA
GCTCTGGCCGACGAG ATCAGCAGCTGAAGC CTGCTCATCTCCAAG
CTCTGGCCGACGAGT TCAGCAGCTGAAGCG TGCTCATCTCCAAGG
TCTGGCCGACGAGTG CAGCAGCTGAAGCGC GCTCATCTCCAAGGC
CTGGCCGACGAGTGG AGCAGCTGAAGCGCC CTCATCTCCAAGGCC
TGGCCGACGAGTGGA GCAGCTGAAGCGCCT TCATCTCCAAGGCCG
GGCCGACGAGTGGAG CAGCTGAAGCGCCTG CATCTCCAAGGCCGA
GCCGACGAGTGGAGA AGCTGAAGCGCCTGG ATCTCCAAGGCCGAG
CCGACGAGTGGAGAA GCTGAAGCGCCTGGA TCTCCAAGGCCGAGG
CGACGAGTGGAGAAA CTGAAGCGCCTGGAG CTCCAAGGCCGAGGA
GACGAGTGGAGAAAT TGAAGCGCCTGGAGA TCCAAGGCCGAGGAC
ACGAGTGGAGAAATC GAAGCGCCTGGAGAA CCAAGGCCGAGGACT
CGAGTGGAGAAATCT AAGCGCCTGGAGAAC CAAGGCCGAGGACTA
GAGTGGAGAAATCTG AGCGCCTGGAGAACT AAGGCCGAGGACTAC
AGTGGAGAAATCTGC GCGCCTGGAGAACTG AGGCCGAGGACTACC
GTGGAGAAATCTGCG CGCCTGGAGAACTGC GGCCGAGGACTACCG
TGGAGAAATCTGCGG GCCTGGAGAACTGCA GCCGAGGACTACCGC
GGAGAAATCTGCGGG CCTGGAGAACTGCAC CCGAGGACTACCGCA
GAGAAATCTGCGGGC CTGGAGAACTGCACG CGAGGACTACCGCAG
AGAAATCTGCGGGCC TGGAGAACTGCACGG GAGGACTACCGCAGC
GAAATCTGCGGGCCA GGAGAACTGCACGGT AGGACTACCGCAGCT
AAATCTGCGGGCCAG GAGAACTGCACGGTG GGACTACCGCAGCTA

	GACTACCGCAGCTAC	GTTCCGAGTGGCTGG	TCCGCGGCTGGAAAC
	ACTACCGCAGCTACC	TTCCGAGTGGCTGGC	CCGCGGCTGGAAACT
	CTACCGCAGCTACCG	TCCGAGTGGCTGGCC	CGCGGCTGGAAACTC
	TACCGCAGCTACCGC	CCGAGTGGCTGGCCT	GCGGCTGGAAACTCT
5	ACCGCAGCTACCGCT	CGAGTGGCTGGCCTC	CGGCTGGAAACTCTT
	CCGCAGCTACCGCTT	GAGTGGCTGGCCTCG	GGCTGGAAACTCTTC
	CGCAGCTACCGCTTC	AGTGGCTGGCCTCGA	GCTGGAAACTCTTCT
	GCAGCTACCGCTTCC	GTGGCTGGCCTCGAG	CTGGAAACTCTTCTA
	CAGCTACCGCTTCCC	TGGCTGGCCTCGAGA	TGGAAACTCTTCTAC
10	AGCTACCGCTTCCCC	GGCTGGCCTCGAGAG	GGAAACTCTTCTACA
	GCTACCGCTTCCCCA	GCTGGCCTCGAGAGC	GAAACTCTTCTACAA
	CTACCGCTTCCCCAA	CTGGCCTCGAGAGCC	AAACTCTTCTACAAC
	TACCGCTTCCCCAAG	TGGCCTCGAGAGCCT	AACTCTTCTACAACT
	ACCGCTTCCCCAAGC	GGCCTCGAGAGCCTC	ACTCTTCTACAACTA
15	CCGCTTCCCCAAGCT	GCCTCGAGAGCCTCG	CTCTTCTACAACTAC
	CGCTTCCCCAAGCTC	CCTCGAGAGCCTCGG	TCTTCTACAACTACG
	GCTTCCCCAAGCTCA	CTCGAGAGCCTCGGA	CTTCTACAACTACGC
	CTTCCCCAAGCTCAC	TCGAGAGCCTCGGAG	TTCTACAACTACGCC
	TTCCCCAAGCTCACG	CGAGAGCCTCGGAGA	TCTACAACTACGCCC
20	TCCCCAAGCTCACGG	GAGAGCCTCGGAGAC	CTACAACTACGCCCT
	CCCCAAGCTCACGGT	AGAGCCTCGGAGACC	TACAACTACGCCCTG
	CCCAAGCTCACGGTC	GAGCCTCGGAGACCT	ACAACTACGCCCTGG
	CCAAGCTCACGGTCA	AGCCTCGGAGACCTC	CAACTACGCCCTGGT
	CAAGCTCACGGTCAT	GCCTCGGAGACCTCT	AACTACGCCCTGGTC
25	AAGCTCACGGTCATT	CCTCGGAGACCTCTT	ACTACGCCCTGGTCA
	AGCTCACGGTCATTA	CTCGGAGACCTCTTC	CTACGCCCTGGTCAT
	GCTCACGGTCATTAC	TCGGAGACCTCTTCC	TACGCCCTGGTCATC
	CTCACGGTCATTACC	CGGAGACCTCTTCCC	ACGCCCTGGTCATCT
	TCACGGTCATTACCG	GGAGACCTCTTCCCC	CGCCCTGGTCATCTT
30	CACGGTCATTACCGA	GAGACCTCTTCCCCA	GCCCTGGTCATCTTC
	ACGGTCATTACCGAG	AGACCTCTTCCCCAA	CCCTGGTCATCTTCG
	CGGTCATTACCGAGT	GACCTCTTCCCCAAC	CCTGGTCATCTTCGA
	GGTCATTACCGAGTA	ACCTCTTCCCCAACC	CTGGTCATCTTCGAG
	GTCATTACCGAGTAC	CCTCTTCCCCAACCT	TGGTCATCTTCGAGA
35	TCATTACCGAGTACT	CTCTTCCCCAACCTC	GGTCATCTTCGAGAT
	CATTACCGAGTACTT	TCTTCCCCAACCTCA	GTCATCTTCGAGATG
	ATTACCGAGTACTTG	CTTCCCCAACCTCAC	TCATCTTCGAGATGA
	TTACCGAGTACTTGC	TTCCCCAACCTCACG	CATCTTCGAGATGAC
	TACCGAGTACTTGCT	TCCCCAACCTCACGG	ATCTTCGAGATGACC
40	ACCGAGTACTTGCTG	CCCCAACCTCACGGT	TCTTCGAGATGACCA
	CCGAGTACTTGCTGC	CCCAACCTCACGGTC	CTTCGAGATGACCAA
	CGAGTACTTGCTGCT	CCAACCTCACGGTCA	TTCGAGATGACCAAT
	GAGTACTTGCTGCTG	CAACCTCACGGTCAT	TCGAGATGACCAATC
	AGTACTTGCTGCTGT	AACCTCACGGTCATC	CGAGATGACCAATCT
45	GTACTTGCTGCTGTT	ACCTCACGGTCATCC	GAGATGACCAATCTC
	TACTTGCTGCTGTTC	CCTCACGGTCATCCG	AGATGACCAATCTCA
	ACTTGCTGCTGTTCC	CTCACGGTCATCCGC	GATGACCAATCTCAA
	CTTGCTGCTGTTCCG	TCACGGTCATCCGCG	ATGACCAATCTCAAG
	TTGCTGCTGTTCCGA	CACGGTCATCCGCGG	TGACCAATCTCAAGG
50	TGCTGCTGTTCCGAG	ACGGTCATCCGCGGC	GACCAATCTCAAGGA
	GCTGCTGTTCCGAGT	CGGTCATCCGCGGCT	ACCAATCTCAAGGAT
	CTGCTGTTCCGAGTG	GGTCATCCGCGGCTG	CCAATCTCAAGGATA
	TGCTGTTCCGAGTGG	GTCATCCGCGGCTGG	CAATCTCAAGGATAT
	GCTGTTCCGAGTGGC	TCATCCGCGGCTGGA	AATCTCAAGGATATT
55	CTGTTCCGAGTGGCT	CATCCGCGGCTGGAA	ATCTCAAGGATATTG
	TGTTCCGAGTGGCTG	ATCCGCGGCTGGAAA	TCTCAAGGATATTGG

	CTCAAGGATATTGGG	TGAGAAAAATGCTGA	ATGCGGTGTCCAATA
	TCAAGGATATTGGGC	GAGAAAAATGCTGAC	TGCGGTGTCCAATAA
	CAAGGATATTGGGCT	AGAAAAATGCTGACC	GCGGTGTCCAATAAC
	AAGGATATTGGGCTT	GAAAAATGCTGACCT	CGGTGTCCAATAACT
5	AGGATATTGGGCTTT	AAAAATGCTGACCTC	GGTGTCCAATAACTA
	GGATATTGGGCTTTA	AAAATGCTGACCTCT	GTGTCCAATAACTAC
	GATATTGGGCTTTAC	AAATGCTGACCTCTG	TGTCCAATAACTACA
	ATATTGGGCTTTACA	AATGCTGACCTCTGT	GTCCAATAACTACAT
	TATTGGGCTTTACAA	ATGCTGACCTCTGTT	TCCAATAACTACATT
10	ATTGGGCTTTACAAC	TGCTGACCTCTGTTA	CCAATAACTACATTG
	TTGGGCTTTACAACC	GCTGACCTCTGTTAC	CAATAACTACATTGT
	TGGGCTTTACAACCT	CTGACCTCTGTTACC	AATAACTACATTGTG
	GGGCTTTACAACCTG	TGACCTCTGTTACCT	ATAACTACATTGTGG
	GGCTTTACAACCTGA	GACCTCTGTTACCTC	TAACTACATTGTGGG
15	GCTTTACAACCTGAG	ACCTCTGTTACCTCT	AACTACATTGTGGGG
	CTTTACAACCTGAGG	CCTCTGTTACCTCTC	ACTACATTGTGGGGA
	TTTACAACCTGAGGA	CTCTGTTACCTCTCC	CTACATTGTGGGGAA
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EXAMPLE 9

INHIBITION OF IGF-I BINDING BY ANTISENSE OLIGONUCLEOTIDES TO IGF-I RECEPTOR

Sub-confluent HaCaT cells were treated as described above with phosphorothioate oligonucleotides IGFR.AS (antisense: 5'-ATCTCTCCGCTTCCTTTC-3'; [SEQ ID NO. 10]; ref 13) and IGFR.S (sense control: 5'-GAAAGGAAGCGGAGAGAT-3'; [SEQ ID NO. 11]; ref 13) IGF-I binding to the cell monolayers was then measured as ^{125}I-IGF-I.

EXAMPLE 10

INHIBITION OF IGFBP-3 PRODUCTION USING ANTISENSE OLIGONUCLEOTIDES

The results of this experiment are shown in Figures 7 and 8.

HaCaT cells were initially plated in DMEM with 10% v/v serum, then AS oligo experiments were performed in complete "Keratinocyte-SFM" (Gibco) to exclude the influence of exogenous IGFBPs. Oligos were synthesised as phosphorothioate (nuclease-resistant) derivatives (Bresatec, South Australia) and were as follows: antisense: AS2, 5'-GCGCCCGCTGCATGACGCCTGCAAC-3' (IGFBP-3 start codon); controls:

AS2NS, 5'-CGGAGATGCCGCATGCCAGCGCAGG-3'; AS4, 5'-AGGCGGCTGACGGCACTA-3'; AS4NS, 5'-GACAGCGTCGGAGCGATC-3'; IGFRAS, 5'-ATCTCTCCGCTTCCTTTC-3'; IGFRS, 5'-GAAAGGAAGCGGAGAGAT-3'. Oligos to IGFBP-3 were based on the published sequence of Spratt *et al* [12]. AS oligos were added to HaCaT monolayers in 0.5ml medium in 24-well plates at the concentrations and addition frequencies indicated. IGFBP-3 measured in cell-conditioned medium using a dot-blot assay, adapted from the Western ligand blot method of Hossenlopp *et al* [11], in which 100µl of conditioned medium was applied to nitrocellulose filters with a vacuum dot-blot apparatus. After drying the membranes at 37°C, relative amounts of IGFBP are determined by ^{125}I-IGF-I-binding, autoradiography and computerised imaging densitometry. Triplicate wells (except in Figure 7, where duplicate wells were measured as shown) were analysed and corrected for changes in cell number per well. Relative cell number per well was determined using an amido black dye method, developed specifically for cultured monolayers of HaCaT cells (14). Cell numbers differed by less than 10% after treatment. For oligos to the IGF receptor, receptor quantitation in intact HaCaT monolayers was by overnight incubation with ^{125}I-IGF-I (30,000cpm/well) at 4°C.

EXAMPLE 11

INHIBITION OF IGFBP-2 PRODUCTION USING RIBOZYMES

Experiments involving ribozymes are generally conducted as described in Internaitonal Patent Application No. WO 89/05852 and in Haselhoff and Gerlach [8]. Ribozymes are constructed with a hybridising region which is complementary in nucleotide sequence to at least part of a target RNA which, in this case, encodes IGFBP-2. Activity of ribozymes is measurable on, for example, Northern blots or using animal models such as in the nude mouse model (15; 16) or the "flaky skin" mouse model (17; 18).

EXAMPLE 12

INHIBITION OF IGFBP-3 PRODUCTION USING RIBOZYMES

The methods described in Example 11 are used for the screening of ribozymes which inhibit IGFBP-3 production. The activity of the ribozymes is determined as in Example 11.

EXAMPLE 13

INHIBITION OF IGF-1 PRODUCTION USING RIBOZYMES

The methods described in Example 11 are used for the screening of ribozymes which inhibit IGF-1 production. The activity of the ribozymes is determined as in Example 11.

EXAMPLE 14

INHIBITION OF IGF-1 RECEPTOR PRODUCTION USING RIBOZYMES

The methods described in Example 11 are used for the screening of ribozymes which inhibit IGF-1 production. The activity of the ribozymes is determined as in Example 11.

Those skilled in the art will appreciate that the invention described herein is susceptible to variations and modifications other than those specifically described. It is to be understood that the invention includes all such variations and modifications. The invention also includes all of the steps, features, compositions and compounds referred to or indicated in this specification, individually or collectively, and any and all combinations of any two or more of said steps or features.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT (countries other than US): ROYAL CHILDREN'S HOSPITAL RESEARCH FOUNDATION
(US only): George A WERTHER and Christopher J WRAIGHT

(ii) TITLE OF INVENTION: A METHOD FOR THE PROPHYLAXIS AND/OR TREATMENT OF PROLIFERATIVE AND/OR INFLAMMATORY SKIN DISORDERS

(iii) NUMBER OF SEQUENCES: 11

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(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT INTERNATIONAL
(B) FILING DATE: 06-JUL-1995

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: PM6725/94
(B) FILING DATE: 08-JUL-1994

(viii) ATTORNEY/AGENT INFORMATION:
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(ix) TELECOMMUNICATION INFORMATION:
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(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 1433 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

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CTGCCGAGAG TGGGCTGCCC CGCGCTGCCG CTGCCGCCGC CGCCGCTGCT GCCGCTGCTG 180

CCGCTGCTGC TGCTGCTACT GGGCGCGAGT GGCGGCGGCG GCGGGGCGCG CGCGGAGGTG 240

CTGTTCCGCT GCCCGCCCTG CACACCCGAG CGCCTGGCCG CCTGCGGGCC CCCGCCGGTT 300

GCGCCGCCCG CCGCGGTGGC CGCAGTGGCC GGAGGCGCCC GCATGCCATG CGCGGAGCTC 360

GTCCGGGAGC CGGGCTGCGG CTGCTGCTCG GTGTGCGCCC GGCTGGAGGG CGAGGCGTGC 420

GGCGTCTACA CCCCGCGCTG CGGCCAGGGG CTGCGCTGCT ATCCCCACCC GGGCTCCGAG 480

CTGCCCCTGC AGGCGCTGGT CATGGGCGAG GGCACTTGTG AGAAGCGCCG GGACGCCGAG 540

TATGGCGCCA GCCCGGAGCA GGTTGCAGAC AATGGCGATG ACCACTCAGA AGGAGGCCTG 600

GTGGAGAACC ACGTGGACAG CACCATGAAC ATGTTGGGCG GGGGAGGCAG TGCTGGCCGG 660

AAGCCCCTCA AGTCGGGTAT GAAGGAGCTG GCCGTGTTCC GGGAGAAGGT CACTGAGCAG 720

CACCGGCAGA TGGGCAAGGG TGGCAAGCAT CACCTTGGCC TGGAGGAGCC CAAGAAGCTG 780

CGACCACCCC CTGCCAGGAC TCCCTGCCAA CAGGAACTGG ACCAGGTCCT GGAGCGGATC 840

TCCACCATGC GCCTTCCGGA TGAGCGGGGC CCTCTGGAGC ACCTCTACTC CCTGCACATC 900

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CAGCGTGGGG AGTGCTGGTG TGTGAACCCC AACACCGGGA AGCTGATCCA GGGAGCCCCC 1020

ACCATCCGGG GGGACCCCGA GTGTCATCTC TTCTACAATG AGCAGCAGGA GGCTTGCGGG 1080

GTGCACACCC AGCGGATGCA GTAGACCGCA GCCAGCCGGT GCCTGGCGCC CCTGCCCCCC 1140

GCCCCTCTCC AAACACCGGC AGAAAACGGA GAGTGCTTGG GTGGTGGGTG CTGGAGGATT 1200

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TTTATTTTTG AACCCCTGTG TCCCTTTTGC ATAAGATTAA AGGAAGGAAA AGT 1433

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 2474 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

CTCAGCGCCC AGCCGCTTCC TGCCTGGATT CCACAGCTTC GCGCCGTGTA CTGTCGCCCC 60

ATCCCTGCGC GCCCAGCCTG CCAAGCAGCG TGCCCCGGTT GCAGGCGTCA TGCAGCGGGC 120

GCGACCCACG CTCTGGGCCG CTGCGCTGAC TCTGCTGGTG CTGCTCCGCG GGCCGCCGGT 180

GGCGCGGGCT GGCGCGAGCT CGGGGGGCTT GGGTCCCGTG GTGCGCTGCG AGCCGTGCGA 240

CGCGCGTGCA CTGGCCCAGT GCGCGCCTCC GCCCGCCGTG TGCGCGGAGC TGGTGCGCGA 300

GCCGGGCTGC GGCTGCTGCC TGACGTGCGC ACTGAGCGAG GGCCAGCCGT GCGGCATCTA 360

CACCGAGCGC TGTGGCTCCG GCCTTCGCTG CCAGCCGTCG CCCGACGAGG CGCGACCGCT 420

GCAGGCGCTG CTGGACGGCC GCGGGCTCTG CGTCAACGCT AGTGCCGTCA GCCGCCTGCG 480

CGCCTACCTG CTGCCAGCGC CGCCAGCTCC AGGAAATGCT AGTGAGTCGG AGGAAGACCG 540

CAGCGCCGGC AGTGTGGAGA GCCCGTCCGT CTCCAGCACG CACCGGGTGT CTGATCCCAA 600

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GCGCTACAAA GTTGACTACG AGTCTCAGAG CACAGATACC CAGAACTTCT CCTCCGAGTC 720

CAAGCGGGAG ACAGAATATG GTCCCTGCCG TAGAGAAATG GAAGACACAC TGAATCACCT 780

GAAGTTCCTC AATGTGCTGA GTCCCAGGGG TGTACACATT CCCAACTGTG ACAAGAAGGG 840

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GCGTGGATCC CTCAACCAAG AAGAATGTTT ATGTCTTCAA GTGACCTGTA CTGCTTGGGG 1560

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GAGGGACAGA GAGACGGGAG AGTCAGCCTC CACATTCAGA GGCATCACAA GTAATGGCAC 1800

AATTCTTCGG ATGACTGCAG AAAATAGTGT TTTGTAGTTC AACAACTCAA GACGAAGCTT 1860

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CCTTAGCACA ATGTAAAAAA GAATAGTAAT ATCAGAACAG GAAGGAGGAA TGGCTTGCTG 1980

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AAGTTTTTAC CATT 2474

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 4989 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

TTTTTTTTTT TTTTGAGAAA GGGAATTTCA TCCCAAATAA AAGGAATGAA GTCTGGCTCC 60

GGAGGAGGGT CCCCGACCTC GCTGTGGGGG CTCCTGTTTC TCTCCGCCGC GCTCTCGCTC 120

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CTGAAGCGCC TGGAGAACTG CACGGTGATC GAGGGCTACC TCCACATCCT GCTCATCTCC 240

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TGAACCGGC 4989

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCGCCCGCTG CATGACGCCT GCAAC 25

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CGGGCGGCTC ACCTGGAGCT GGCG 24

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

AGGCGGCTGA CGGCACTA 18

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 19 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

CAGGCGTCAT GCAGCGGGC 19

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 25 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

CGGAGATGCC GCATGCCAGC GCAGG 25

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

GACAGCGTCG GAGCGATC 18

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

ATCTCTCCGC TTCCTTTC 18

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 base pairs
(B) TYPE: nucleic acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

GAAAGGAAGC GGAGAGAT 18

CLAIMS:

1. A method for ameliorating the effects of a proliferative and/or inflammatory skin disorder in a mammal, said method comprising contacting the proliferating and/or inflamed skin or skin capable of proliferation and/or inflammation with an effective amount of a nucleic acid molecule or chemical analogue thereof capable of inhibiting or otherwise reducing growth factor mediated cell proliferation and/or inflammation.

2. A method according to claim 1 wherein cell proliferation and/or inflammation is mediated by at least one of insulin-like growth factor I (IGF-I), keratinocyte growth factor (KGF), transforming growth factor-α (TGFα), tumour necrosis factor-α (TNFα), interleukin (IL) -1 (IL-1), IL-4, IL-6, IL-8 and/or basic fibroblast growth factor (bFGF).

3. A method according to claim 2 wherein cell proliferation and/or inflammation is mediated by IGF-I.

4. A method according to claim 1 wherein the nucleic acid molecule inhibits or otherwise reduces IGF-I mediated cell proliferation and/or inflammation.

5. A method according to claim 1 wherein the proliferative or inflammatory skin disorder is psoriasis, ichthyosis, pityriasis, rubra, pilaris, serborrhoea, keloids, keratosis, neoplasias, scleroderma, warts, benign growths or cancers of the skin.

6. A method according to claim 5 wherein the skin condition is psoriasis.

7. A method according to claim 1 or 4 or 6 wherein the mammal is a human.

8. A method according to claim 1 or 4 or 6 wherein the nucleic acid molecule is capable of inhibiting, reducing or otherwise interfering with IGF-I-interaction with its receptor.

9. A method according to claim 8 wherein the nucleic acid molecule is an antisense molecule capable of reducing expression of a gene encoding IGF-I, IGF-I-receptor or an IGF binding protein (IGFBP).

10. A method according to claim 9 wherein the nucleic acid molecule is an antisense molecule capable of reducing expression of a gene encoding IGFBP-2, -3, -4, -5 or -6.

11. A method according to claim 10 wherein the nucleic acid molecule is an antisense molecule capable of reducing expression of a gene encoding IGFBP-2 or IGFBP-3.

12. A method according to claim 11 wherein the antisense molecule is at least about 15 nucleotides in length and is capable of interacting with at least one sequence selected from the list set forth in Example 6 or Example 7.

13. A method according to claim 11 wherein the antisense molecule comprises the nucleotide sequence:

5'-ATCTCTCCGCTTCCTTTC-3' [SEQ ID NO:10].

14. A nucleic acid molecule comprising at least about 10 nucleotides capable of hybridising to or forming a heteroduplex or otherwise interacting with an RNA molecule directed from a gene corresponding to a genomic form of SEQ ID NO:1 and/or SEQ ID NO:2 and which thereby reduces or inhibits translation of said RNA molecule.

15. A nucleic acid molecule according to claim 14 wherein said molecule comprises at least about 15 nucleotides.

16. A nucleic acid molecule according to claim 15 wherein said molecule is capable of interacting with at least one nucleotide sequence selected from the list set forth in Example 6 and Example 7.

17. A nucleic acid molecule according to claim 15 or 16 comprising the nucleotide sequence:

5'-ATCTCTCCGCTTCCTTTC-3' [SEQ ID NO:10].

18. A method of ameliorating the effects of psoriasis, said method comprising contacting proliferating skin or skin capable of proliferation with an effective amount of one or more nucleic acid molecules or chemical analogues thereof capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation wherein said one or more molecules comprises a polynucleotide capable of interacting with mRNA directed from an IGF-I gene, an IGF-I receptor gene or a gene encoding an IGFBP.

19. A method according to claim 18 wherein the IGFBP is IGFBP-2 or IGFBP-3.

20. A method according to claim 18 or 19 wherein the mammal is a human.

21. A method according to claim 20 wherein the nucleic acid molecule is capable of interacting with a nucleotide sequence selected from the list set forth in Example 6 or Example 7.

22. A method according to claim 18 wherein the nucleic acid molecule comprises the nucleotide sequence:

5'-ATCTCTCCGCTTCCTTTC-3' [SEQ ID NO:10].

23. A pharmaceutical composition for topical administration said composition comprising a nucleic acid molecule capable of inhibiting or otherwise reducing IGF-I mediated cell proliferation said composition further comprising one or more pharmaceutically acceptable carriers and/or diluents.

24. A pharmaceutical composition according to claim 23 wherein the nucleic acid molecule is an antisense molecule to a gene encoding IGF-I, IGF-I-receptor or an IGFBP.

25. A pharmaceutical composition according to claim 24 wherein the nucleic acid molecule is capable of targeting a gene encoding IGFBP-2 and/or IGFBP-3.

26. A pharmaceutical composition according to claim 24 capable of interacting with at least one nucleotide sequence set forth in Example 6 or Example 7.

27. Use of a nucleic acid molecule in the manufacture of a medicament for the treatment of a proliferative and/or inflammatory skin disorder mediated by IGF-I.

28. Use according to claim 27 wherein the skin disorder is psoriasis.

29. A ribozyme comprising a hybridising region and a catalytic region wherein the hybridising region is capable of hybridising to at least part of a target mRNA sequence transcribed from a genomic gene corresponding to SEQ ID NO:1 or SEQ ID NO:2 wherein said catalytic domain is capable of cleaving said target mRNA sequence to reduce or inhibit IGF-I mediated cell proliferation or inflammation.

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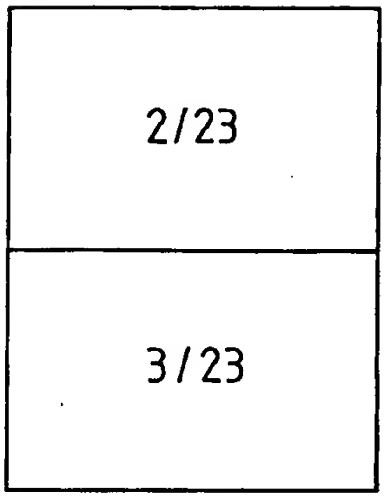

FIG 1

FIGURE 1

```
1      ATTCGGGGCG AGGGAGGAGG AAGAAGCGGA GGAGGCGGCT CCCGCTCGCA
51     GGGCCGTGCA CCTGCCCGCC CGCCCGCTCG CTCGCTCGCC CGCCGCGCCG
101    CGCTGCCGAC CGCCAGCATG CTGCCGAGAG TGGGCTGCCC CGCGCTGCCG
151    CTGCCGCCGC CGCCGCTGCT GCCGCTGCTG CCGCTGCTGC TGCTGCTACT
201    GGGCGCGAGT GGCGGCGGCG GCGGGGCGCG CGCGGAGGTG CTGTTCCGCT
251    GCCCGCCCTG CACACCCGAG CGCCTGGCCG CCTGCGGGCC CCCGCCGGTT
301    GCGCCGCCCG CCGCGGTGGC CGCAGTGGCC GGAGGCGCCC GCATGCCATG
351    CGCGGAGCTC GTCCGGGAGC CGGGCTGCGG CTGCTGCTCG GTGTGCGCCC
401    GGCTGGAGGG CGAGGCGTGC GGCGTCTACA CCCCGCGCTG CGGCCAGGGG
451    CTGCGCTGCT ATCCCCACCC GGGCTCCGAG CTGCCCCTGC AGGCGCTGGT
501    CATGGGCGAG GGCACTTGTG AGAAGCGCCG GGACGCCGAG TATGGCGCCA
551    GCCCGGAGCA GGTTGCAGAC AATGGCGATG ACCACTCAGA AGGAGGCCTG
601    GTGGAGAACC ACGTGGACAG CACCATGAAC ATGTTGGGCG GGGGAGGCAG
651    TGCTGGCCGG AAGCCCCTCA AGTCGGGTAT GAAGGAGCTG GCCGTGTTCC
701    GGGAGAAGGT CACTGAGCAG CACCGGCAGA TGGGCAAGGG TGGCAAGCAT
```

FIGURE 1 (continued...)

```
751    CACCTTGGCC TGGAGGAGCC CAAGAAGCTG CGACCACCCC CTGCCAGGAC
801    TCCCTGCCAA CAGGAACTGG ACCAGGTCCT GGAGCGGATC TCCACCATGC
851    GCCTTCCGGA TGAGCGGGGC CCTCTGGAGC ACCTCTACTC CCTGCACATC
901    CCCAACTGTG ACAAGCATGG CCTGTACAAC CTCAAACAGT GCAAGATGTC
951    TCTGAACGGG CAGCGTGGGG AGTGCTGGTG TGTGAACCCC AACACCGGGA
1001   AGCTGATCCA GGGAGCCCCC ACCATCCGGG GGGACCCCGA GTGTCATCTC
1051   TTCTACAATG AGCAGCAGGA GGCTTGCGGG GTGCACACCC AGCGGATGCA
1101   GTAGACCGCA GCCAGCCGGT GCCTGGCGCC CCTGCCCCCC GCCCCTCTCC
1151   AAACACCGGC AGAAAACGGA GAGTGCTTGG GTGGTGGGTG CTGGAGGATT
1201   TTCCAGTTCT GACACACGTA TTTATATTTG GAAAGAGACC AGCACCGAGC
1251   TCGGCACCTC CCCGGCCTCT CTCTTCCCAG CTGCAGATGC CACACCTGCT
1301   CCTTCTTGCT TTCCCCGGGG GAGGAAGGGG GTTGTGGTCG GGGAGCTGGG
1351   GTACAGGTTT GGGGAGGGGG AAGAGAAATT TTTATTTTTG AACCCCTGTG
1401   TCCCTTTTGC ATAAGATTAA AGGAAGGAAA AGT
```

4/23

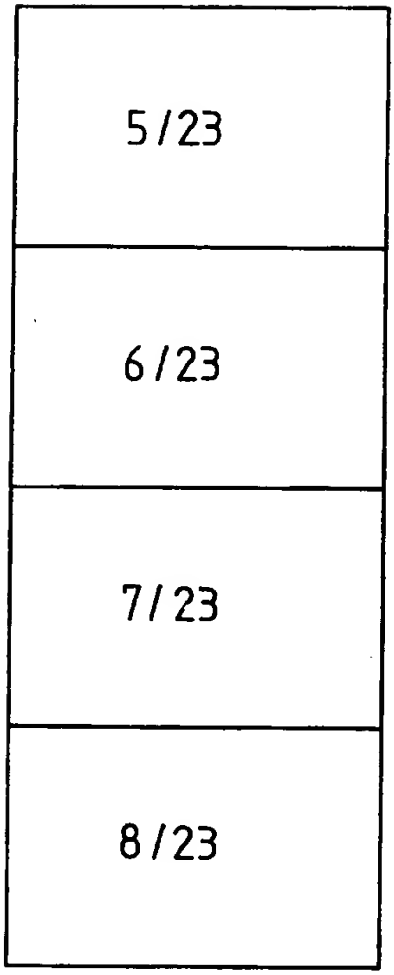

FIG 2

FIGURE 2

```
1      CTCAGCGCCC AGCCGCTTCC TGCCTGGATT CCACAGCTTC GCGCCGTGTA
51     CTGTCGCCCC ATCCCTGCGC GCCCAGCCTG CCAAGCAGCG TGCCCCGGTT
101    GCAGGCGTCA TGCAGCGGGC GCGACCCACG CTCTGGGCCG CTGCGCTGAC
151    TCTGCTGGTG CTGCTCCGCG GGCCGCCGGT GGCGCGGGCT GGCGCGAGCT
201    CGGGGGGCTT GGGTCCCGTG GTGCGCTGCG AGCCGTGCGA CGCGCGTGCA
251    CTGGCCCAGT GCGCGCCTCC GCCCGCCGTG TGCGCGGAGC TGGTGCGCGA
301    GCCGGGCTGC GGCTGCTGCC TGACGTGCGC ACTGAGCGAG GGCCAGCCGT
351    GCGGCATCTA CACCGAGCGC TGTGGCTCCG GCCTTCGCTG CCAGCCGTCG
401    CCCGACGAGG CGCGACCGCT GCAGGCGCTG CTGGACGGCC GCGGGCTCTG
451    CGTCAACGCT AGTGCCGTCA GCCGCCTGCG CGCCTACCTG CTGCCAGCGC
501    CGCCAGCTCC AGGAAATGCT AGTGAGTCGG AGGAAGACCG CAGCGCCGGC
551    AGTGTGGAGA GCCCGTCCGT CTCCAGCACG CACCGGGTGT CTGATCCCAA
601    GTTCCACCCC CTCCATTCAA AGATAATCAT CATCAAGAAA GGGCATGCTA
651    AAGACAGCCA GCGCTACAAA GTTGACTACG AGTCTCAGAG CACAGATACC
701    CAGAACTTCT CCTCCGAGTC CAAGCGGGAG ACAGAATATG GTCCCTGCCG
```

FIGURE 2 (Continued...)

```
751    TAGAGAAATG GAAGACACAC TGAATCACCT GAAGTTCCTC AATGTGCTGA
801    GTCCCAGGGG TGTACACATT CCCAACTGTG ACAAGAAGGG ATTTTATAAG
851    AAAAAGCAGT GTCGCCCTTC CAAAGGCAGG AAGCGGGGCT TCTGCTGGTG
901    TGTGGATAAG TATGGGCAGC CTCTCCCAGG CTACACCACC AAGGGGAAGG
951    AGGACGTGCA CTGCTACAGC ATGCAGAGCA AGTAGACGCC TGCCGCAAGT
1001   TAATGTGGAG CTCAAATATG CCTTATTTTG CACAAAAGAC TGCCAAGGAC
1051   ATGACCAGCA GCTGGCTACA GCCTCGATTT ATATTTCTGT TTGTGGTGAA
1101   CTGATTTTTT TTAAACCAAA GTTTAGAAAG AGGTTTTTGA AATGCCTATG
1151   GTTTCTTTGA ATGGTAAACT TGAGCATCTT TTCACTTTCC AGTAGTCAGC
1201   AAAGAGCAGT TTGAATTTTC TTGTCGCTTC CTATCAAAAT ATTCAGAGAC
1251   TCGAGCACAG CACCCAGACT TCATGCGCCC GTGGAATGCT CACCACATGT
1301   TGGTCGAAGC GGCCGACCAC TGACTTTGTG ACTTAGGCGG CTGTGTTGCC
1351   TATGTAGAGA ACACGCTTCA CCCCCACTCC CCGTACAGTG CGCACAGGCT
1401   TTATCGAGAA TAGGAAAACC TTTAAACCCC GGTCATCCGG ACATCCCAAC
1451   GCATGCTCCT GGAGCTCACA GCCTTCTGTG GTGTCATTTC TGAAACAAGG
```

FIGURE 2 (Continued...)

```
1501   GCGTGGATCC CTCAACCAAG AAGAATGTTT ATGTCTTCAA GTGACCTGTA
1551   CTGCTTGGGG ACTATTGGAG AAAATAAGGT GGAGTCCTAC TTGTTTAAAA
1601   AATATGTATC TAAGAATGTT CTAGGGCACT CTGGGAACCT ATAAAGGCAG
1651   GTATTTCGGG CCCTCCTCTT CAGGAATCTT CCTGAAGACA TGGCCCAGTC
1701   GAAGGCCCAG GATGGCTTTT GCTGCGGCCC CGTGGGGTAG GAGGGACAGA
1751   GAGACGGGAG AGTCAGCCTC CACATTCAGA GGCATCACAA GTAATGGCAC
1801   AATTCTTCGG ATGACTGCAG AAAATAGTGT TTTGTAGTTC AACAACTCAA
1851   GACGAAGCTT ATTTCTGAGG ATAAGCTCTT TAAAGGCAAA GCTTTATTTT
1901   CATCTCTCAT CTTTTGTCCT CCTTAGCACA ATGTAAAAAA GAATAGTAAT
1951   ATCAGAACAG GAAGGAGGAA TGGCTTGCTG GGGAGCCCAT CCAGGACACT
2001   GGGAGCACAT AGAGATTCAC CCATGTTTGT TGAACTTAGA GTCATTCTCA
2051   TGCTTTTCTT TATAATTCAC ACATATATGC AGAGAAGATA TGTTCTTGTT
2101   AACATTGTAT ACAACATAGC CCCAAATATA GTAAGATCTA TACTAGATAA
2151   TCCTAGATGA AATGTTAGAG ATGCTATATG ATACAACTGT GGCCATGACT
2201   GAGGAAAGGA GCTCACGCCC AGAGACTGGG CTGCTCTCCC GGAGGCCAAA
```

FIGURE 2 (Continued...)

```
2251   CCCAAGAAGG TCTGGCAAAG TCAGGCTCAG GGAGACTCTG CCCTGCTGCA
2301   GACCTCGGTG TGGACACACG CTGCATAGAG CTCTCCTTGA AAACAGAGGG
2351   GTCTCAAGAC ATTCTGCCTA CCTATTAGCT TTTCTTTATT TTTTTAACTT
2401   TTTGGGGGGA AAAGTATTTT TGAGAAGTTT GTCTTGCAAT GTATTTATAA
2451   ATAGTAAATA AAGTTTTTAC CATT
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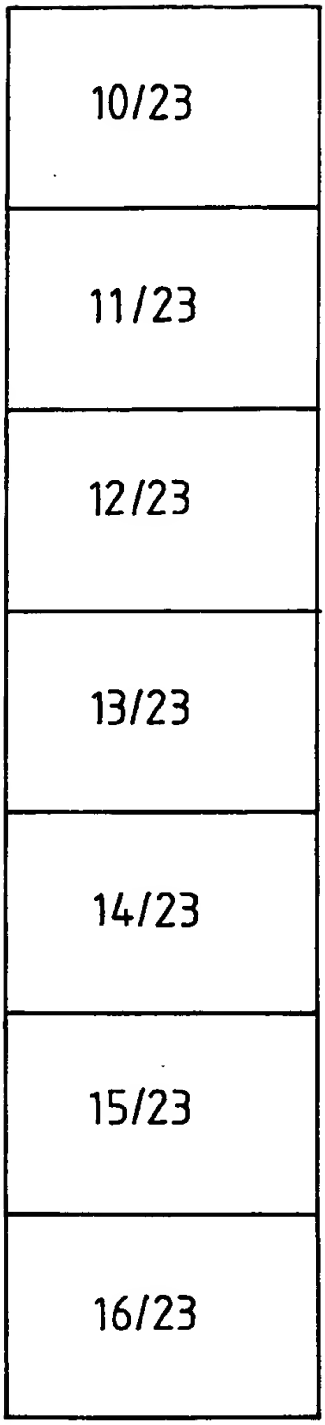

FIG 3

FIGURE 3

```
1     TTTTTTTTTT TTTTGAGAAA GGGAATTTCA TCCCAAATAA AAGGAATGAA
51    GTCTGGCTCC GGAGGAGGGT CCCCGACCTC GCTGTGGGGG CTCCTGTTTC
101   TCTCCGCCGC GCTCTCGCTC TGGCCGACGA GTGGAGAAAT CTGCGGGCCA
151   GGCATCGACA TCCGCAACGA CTATCAGCAG CTGAAGCGCC TGGAGAACTG
201   CACGGTGATC GAGGGCTACC TCCACATCCT GCTCATCTCC AAGGCCGAGG
251   ACTACCGCAG CTACCGCTTC CCCAAGCTCA CGGTCATTAC CGAGTACTTG
301   CTGCTGTTCC GAGTGGCTGG CCTCGAGAGC CTCGGAGACC TCTTCCCCAA
351   CCTCACGGTC ATCCGCGGCT GGAAACTCTT CTACAACTAC GCCCTGGTCA
401   TCTTCGAGAT GACCAATCTC AAGGATATTG GGCTTTACAA CCTGAGGAAC
451   ATTACTCGGG GGGCCATCAG GATTGAGAAA AATGCTGACC TCTGTTACCT
501   CTCCACTGTG GACTGGTCCC TGATCCTGGA TGCGGTGTCC AATAACTACA
551   TTGTGGGGAA TAAGCCCCCA AAGGAATGTG GGGACCTGTG TCCAGGGACC
601   ATGGAGGAGA AGCCGATGTG TGAGAAGACC ACCATCAACA ATGAGTACAA
651   CTACCGCTGC TGGACCACAA ACCGCTGCCA GAAAATGTGC CCAAGCACGT
701   GTGGGAAGCG GGCGTGCACC GAGAACAATG AGTGCTGCCA CCCCGAGTGC
```

FIGURE 3 (Continued...)

```
751    CTGGGCAGCT GCAGCGCGCC TGACAACGAC ACGGCCTGTG TAGCTTGCCG
801    CCACTACTAC TATGCCGGTG TCTGTGTGCC TGCCTGCCCG CCCAACACCT
851    ACAGGTTTGA GGGCTGGCGC TGTGTGGACC GTGACTTCTG CGCCAACATC
901    CTCAGCGCCG AGAGCAGCGA CTCCGAGGGG TTTGTGATCC ACGACGGCGA
951    GTGCATGCAG GAGTGCCCCT CGGGCTTCAT CCGCAACGGC AGCCAGAGCA
1001   TGTACTGCAT CCCTTGTGAA GGTCCTTGCC CGAAGGTCTG TGAGGAAGAA
1051   AAGAAAACAA AGACCATTGA TTCTGTTACT TCTGCTCAGA TGCTCCAAGG
1101   ATGCACCATC TTCAAGGGCA ATTTGCTCAT TAACATCCGA CGGGGGAATA
1151   ACATTGCTTC AGAGCTGGAG AACTTCATGG GGCTCATCGA GGTGGTGACG
1201   GGCTACGTGA AGATCCGCCA TTCTCATGCC TTGGTCTCCT TGTCCTTCCT
1251   AAAAAACCTT CGCCTCATCC TAGGAGAGGA GCAGCTAGAA GGGAATTACT
1301   CCTTCTACGT CCTCGACAAC CAGAACTTGC AGCAACTGTG GGACTGGGAC
1351   CACCGCAACC TGACCATCAA AGCAGGGAAA ATGTACTTTG CTTTCAATCC
1401   CAAATTATGT GTTTCCGAAA TTTACCGCAT GGAGGAAGTG ACGGGGACTA
1451   AAGGGCGCCA AAGCAAAGGG GACATAAACA CCAGGAACAA CGGGGAGAGA
```

FIGURE 3 (Continued...)

```
1501   GCCTCCTGTG AAAGTGACGT CCTGCATTTC ACCTCCACCA CCACGTCGAA
1551   GAATCGCATC ATCATAACCT GGCACCGGTA CCGGCCCCCT GACTACAGGG
1601   ATCTCATCAG CTTCACCGTT TACTACAAGG AAGCACCCTT TAAGAATGTC
1651   ACAGAGTATG ATGGGCAGGA TGCCTGCGGC TCCAACAGCT GGAACATGGT
1701   GGACGTGGAC CTCCCGCCCA ACAAGGACGT GGAGCCCGGC ATCTTACTAC
1751   ATGGGCTGAA GCCCTGGACT CAGTACGCCG TTTACGTCAA GGCTGTGACC
1801   CTCACCATGG TGGAGAACGA CCATATCCGT GGGGCCAAGA GTGAGATCTT
1851   GTACATTCGC ACCAATGCTT CAGTTCCTTC CATTCCCTTG GACGTTCTTT
1901   CAGCATCGAA CTCCTCTTCT CAGTTAATCG TGAAGTGGAA CCCTCCCTCT
1951   CTGCCCAACG GCAACCTGAG TTACTACATT GTGCGCTGGC AGCGGCAGCC
2001   TCAGGACGGC TACCTTTACC GGCACAATTA CTGCTCCAAA GACAAAATCC
2051   CCATCAGGAA GTATGCCGAC GGCACCATCG ACATTGAGGA GGTCACAGAG
2101   AACCCCAAGA CTGAGGTGTG TGGTGGGGAG AAAGGGCCTT GCTGCGCCTG
2151   CCCCAAAACT GAAGCCGAGA AGCAGGCCGA GAAGGAGGAG GCTGAATACC
2201   GCAAAGTCTT TGAGAATTTC CTGCACAACT CCATCTTCGT GCCCAGACCT
```

FIGURE 3 (Continued...)

```
2251   GAAAGGAAGC GGAGAGATGT CATGCAAGTG GCCAACACCA CCATGTCCAG
2301   CCGAAGCAGG AACACCACGG CCGCAGACAC CTACAACATC ACCGACCCGG
2351   AAGAGCTGGA GACAGAGTAC CCTTTCTTTG AGAGCAGAGT GGATAACAAG
2401   GAGAGAACTG TCATTTCTAA CCTTCGGCCT TTCACATTGT ACCGCATCGA
2451   TATCCACAGC TGCAACCACG AGGCTGAGAA GCTGGGCTGC AGCGCCTCCA
2501   ACTTCGTCTT TGCAAGGACT ATGCCCGCAG AAGGAGCAGA TGACATTCCT
2551   GGGCCAGTGA CCTGGGAGCC AAGGCCTGAA AACTCCATCT TTTTAAAGTG
2601   GCCGGAACCT GAGAATCCCA ATGGATTGAT TCTAATGTAT GAAATAAAAT
2651   ACGGATCACA AGTTGAGGAT CAGCGAGAAT GTGTGTCCAG ACAGGAATAC
2701   AGGAAGTATG GAGGGGCCAA GCTAAACCGG CTAAACCCGG GGAACTACAC
2751   AGCCCGGATT CAGGCCACAT CTCTCTCTGG GAATGGGTCG TGGACAGATC
2801   CTGTGTTCTT CTATGTCCAG GCCAAAACAG GATATGAAAA CTTCATCCAT
2851   CTGATCATCG CTCTGCCCGT CGCTGTCCTG TTGATCGTGG GAGGGTTGGT
2901   GATTATGCTG TACGTCTTCC ATAGAAAGAG AAATAACAGC AGGCTGGGGA
2951   ATGGAGTGCT GTATGCCTCT GTGAACCCGG AGTACTTCAG CGCTGCTGAT
```

FIGURE 3 (Continued...)

```
3001   GTGTACGTTC CTGATGAGTG GGAGGTGGCT CGGGAGAAGA TCACCATGAG
3051   CCGGGAACTT GGGCAGGGGT CGTTTGGGAT GGTCTATGAA GGAGTTGCCA
3101   AGGGTGTGGT GAAAGATGAA CCTGAAACCA GAGTGGCCAT TAAAACAGTG
3151   AACGAGGCCG CAAGCATGCG TGAGAGGATT GAGTTTCTCA ACGAAGCTTC
3201   TGTGATGAAG GAGTTCAATT GTCACCATGT GGTGCGATTG CTGGGTGTGG
3251   TGTCCCAAGG CCAGCCAACA CTGGTCATCA TGGAACTGAT GACACGGGGC
3301   GATCTCAAAA GTTATCTCCG GTCTCTGAGG CCAGAAATGG AGAATAATCC
3351   AGTCCTAGCA CCTCCAAGCC TGAGCAAGAT GATTCAGATG GCCGGAGAGA
3401   TTGCAGACGG CATGGCATAC CTCAACGCCA ATAAGTTCGT CCACAGAGAC
3451   CTTGCTGCCC GGAATTGCAT GGTAGCCGAA GATTTCACAG TCAAAATCGG
3501   AGATTTTGGT ATGACGCGAG ATATCTATGA GACAGACTAT TACCGGAAAG
3551   GAGGCAAAGG GCTGCTGCCC GTGCGCTGGA TGTCTCCTGA GTCCCTCAAG
3601   GATGGAGTCT TCACCACTTA CTCGGACGTC TGGTCCTTCG GGGTCGTCCT
3651   CTGGGAGATC GCCACACTGG CCGAGCAGCC CTACCAGGGC TTGTCCAACG
3701   AGCAAGTCCT TCGCTTCGTC ATGGAGGGCG GCCTTCTGGA CAAGCCAGAC
```

FIGURE 3 (Continued...)

```
3751   AACTGTCCTG ACATGCTGTT TGAACTGATG CGCATGTGCT GGCAGTATAA
3801   CCCCAAGATG AGGCCTTCCT TCCTGGAGAT CATCAGCAGC ATCAAAGAGG
3851   AGATGGAGCC TGGCTTCCGG GAGGTCTCCT TCTACTACAG CGAGGAGAAC
3901   AAGCTGCCCG AGCCGGAGGA GCTGGACCTG GAGCCAGAGA ACATGGAGAG
3951   CGTCCCCCTG GACCCCTCGG CCTCCTCGTC CTCCCTGCCA CTGCCCGACA
4001   GACACTCAGG ACACAAGGCC GAGAACGGCC CCGGCCCTGG GGTGCTGGTC
4051   CTCCGCGCCA GCTTCGACGA GAGACAGCCT TACGCCCACA TGAACGGGGG
4101   CCGCAAGAAC GAGCGGGCCT TGCCGCTGCC CCAGTCTTCG ACCTGCTGAT
4151   CCTTGGATCC TGAATCTGTG CAAACAGTAA CGTGTGCGCA CGCGCAGCGG
4201   GGTGGGGGGG GAGAGAGAGT TTTAACAATC CATTCACAAG CCTCCTGTAC
4251   CTCAGTGGAT CTTCAGTTCT GCCCTTGCTG CCCGCGGGAG ACAGCTTCTC
4301   TGCAGTAAAA CACATTTGGG ATGTTCCTTT TTTCAATATG CAAGCAGCTT
4351   TTTATTCCCT GCCCAAACCC TTAACTGACA TGGGCCTTTA AGAACCTTAA
4401   TGACAACACT TAATAGCAAC AGAGCACTTG AGAACCAGTC TCCTCACTCT
4451   GTCCCTGTCC TTCCCTGTTC TCCCTTTCTC TCTCCTCTCT GCTTCATAAC
```

FIGURE 3 (Continued...)

```
4501   GGAAAAATAA TTGCCACAAG TCCAGCTGGG AAGCCCTTTT TATCAGTTTG
4551   AGGAAGTGGC TGTCCCTGTG GCCCCATCCA ACCACTGTAC ACACCCGCCT
4601   GACACCGTGG GTCATTACAA AAAAACACGT GGAGATGGAA ATTTTTACCT
4651   TTATCTTTCA CCTTTCTAGG GACATGAAAT TTACAAAGGG CCATCGTTCA
4701   TCCAAGGCTG TTACCATTTT AACGCTGCCT AATTTTGCCA AAATCCTGAA
4751   CTTTCTCCCT CATCGGCCCG GCGCTGATTC CTCGTGTCCG GAGGCATGGG
4801   TGAGCATGGC AGCTGGTTGC TCCATTTGAG AGACACGCTG GCGACACACT
4851   CCGTCCATCC GACTGCCCCT GCTGTGCTGC TCAAGGCCAC AGGCACACAG
4901   GTCTCATTGC TTCTGACTAG ATTATTATTT GGGGGAACTG GACACAATAG
4951   GTCTTTCTCT CAGTGAAGGT GGGGAGAAGC TGAACCGGC
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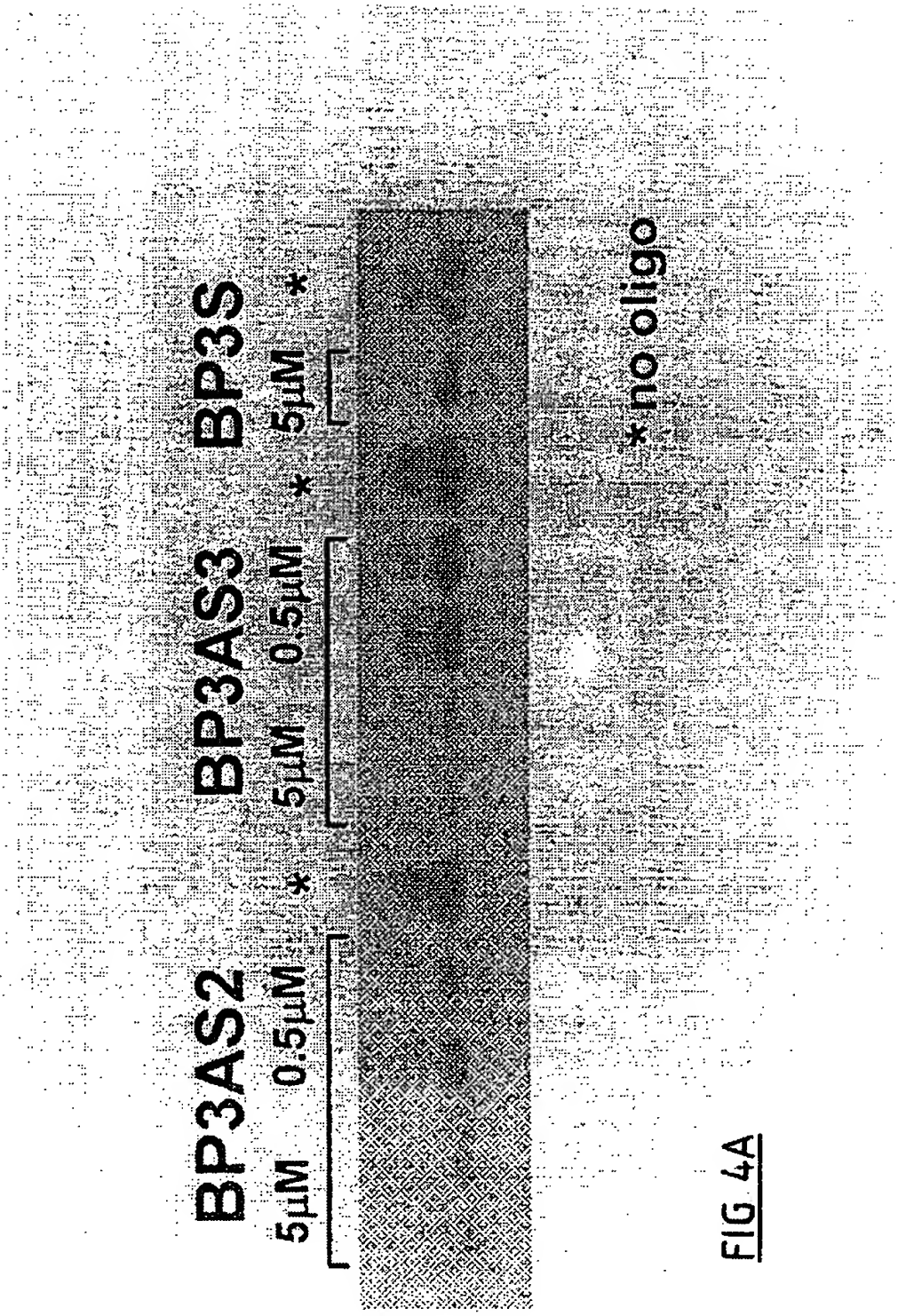


FIG 4A

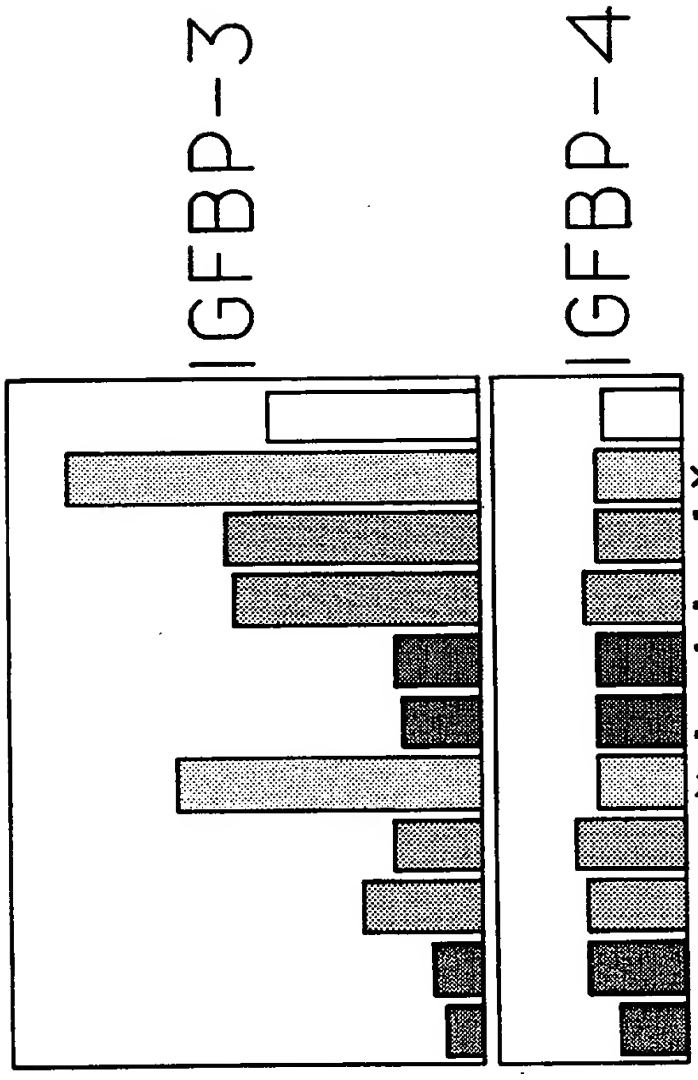


FIG 4B

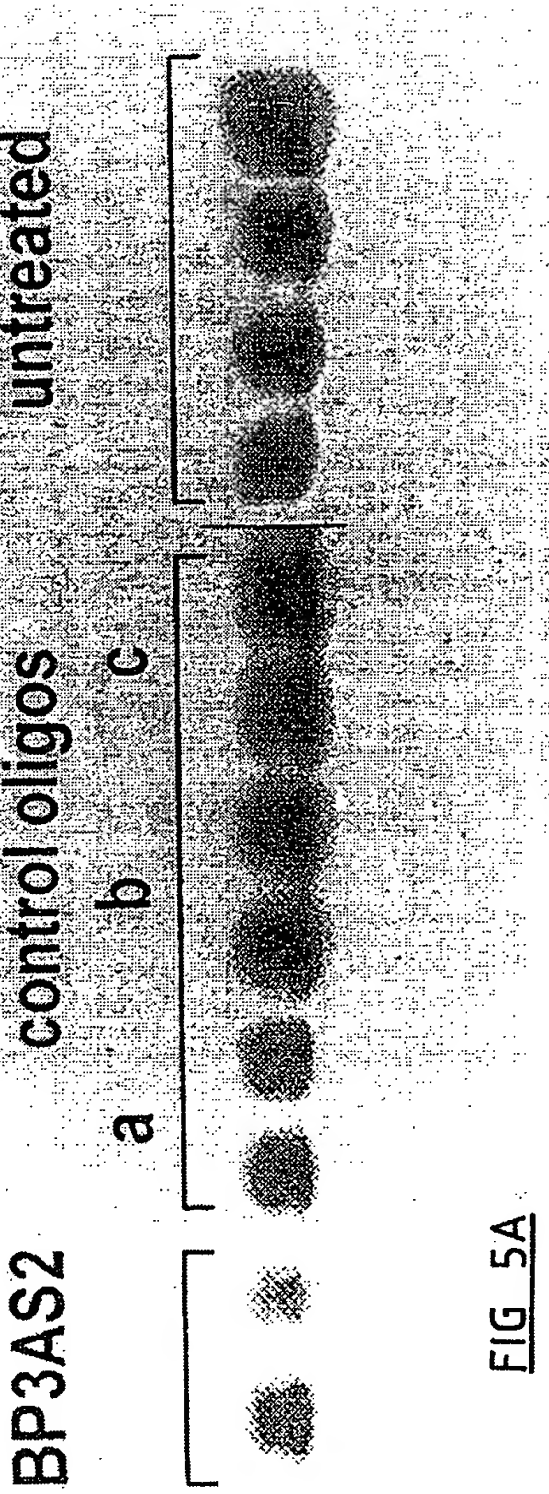


FIG 5A

FIG 5B

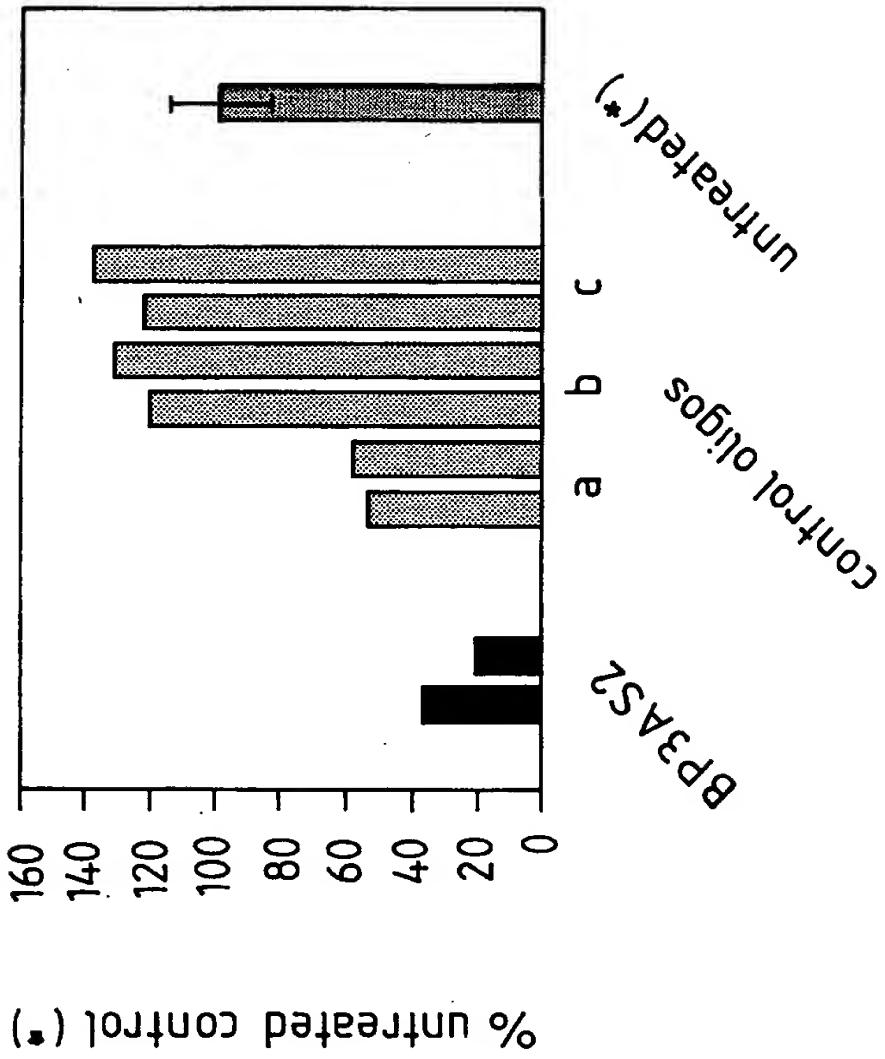

% untreated control (*)
160
140
120
100
80
60
40
20
0
BP3AS2
control oligos
a
b
c
untreated(*)

FIGURE 6 Inhibition of IGF-I binding by antisense oligonucleotides to IGF-I receptor

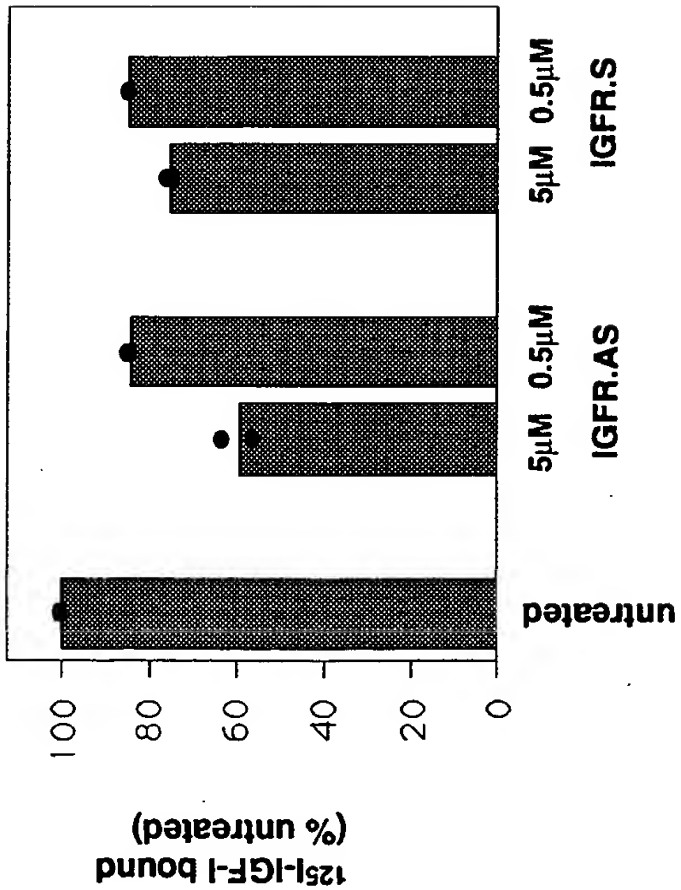
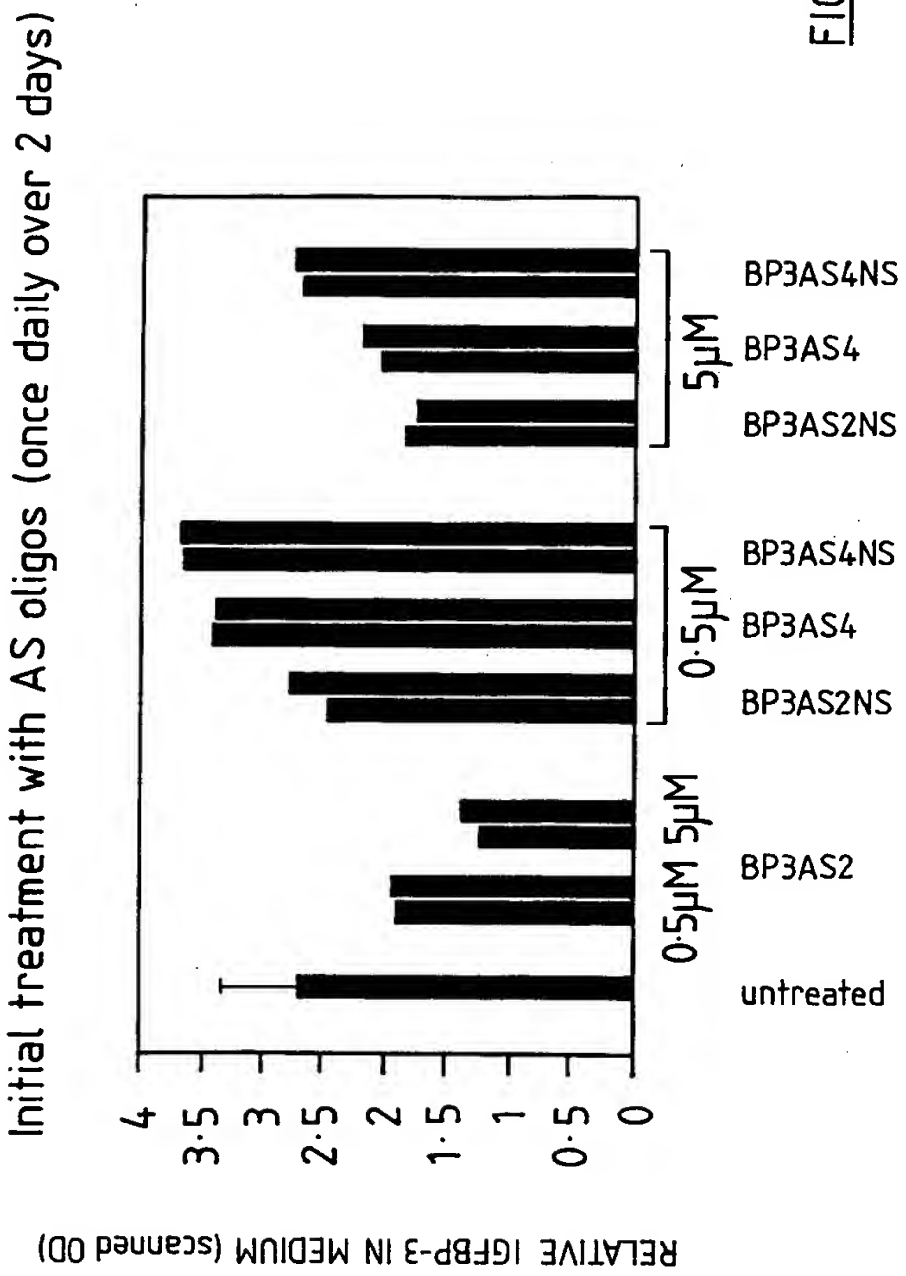


FIG 7


Initial treatment with AS oligos (once daily over 2 days)
RELATIVE IGFBP-3 IN MEDIUM (scanned OD)
4
3·5
3
2·5
2
1·5
1
0·5
0
untreated
BP3AS2
0·5µM 5µM
BP3AS4NS
BP3AS4
BP3AS2NS
0·5µM
BP3AS4NS
BP3AS4
BP3AS2NS
5µM

Optimization of IGFBP-3 AS oligo concentration

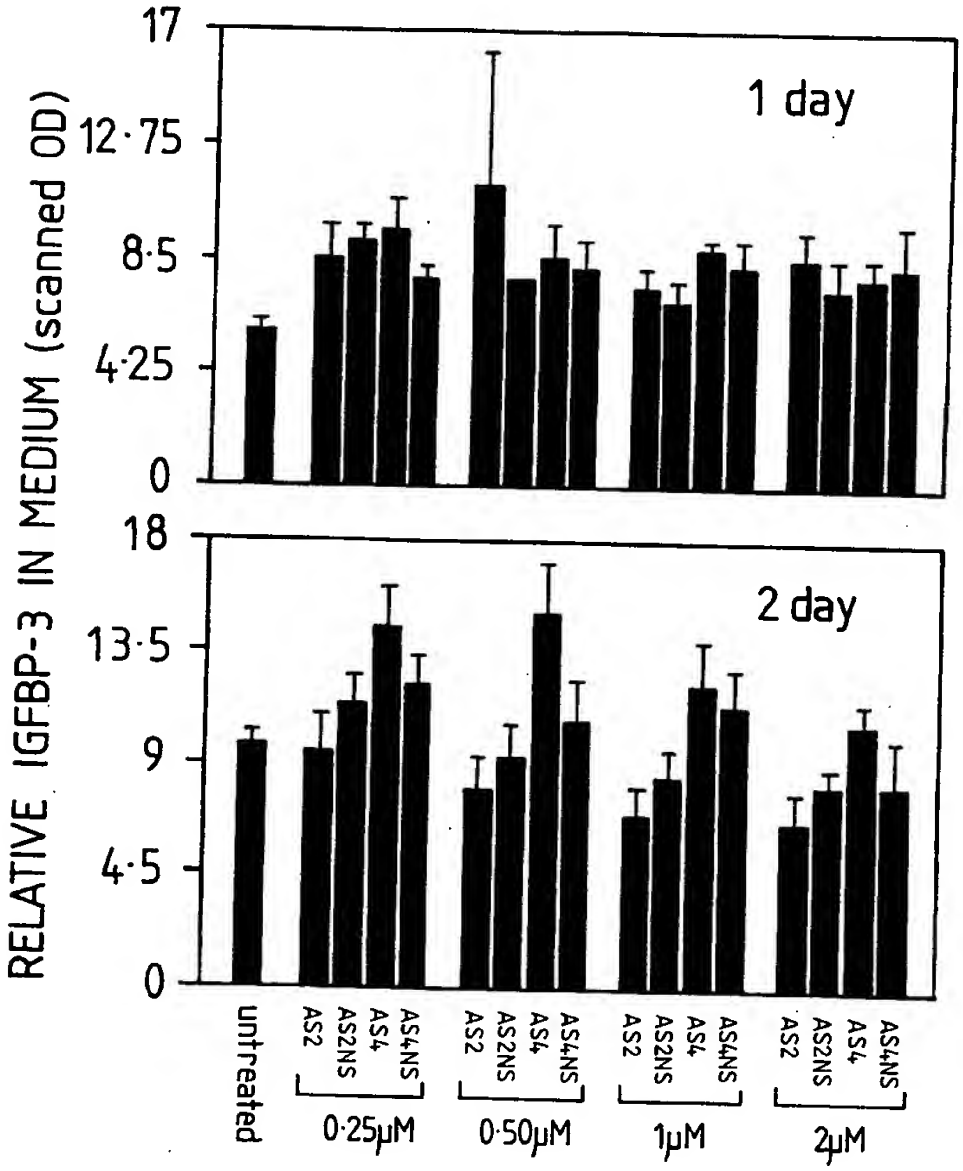


FIG 8

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00410

A. CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]: A61K 31/70, C07K 21/02, C07K 21/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[6] A61K, C07K, C12N
CHEMICAL ABSTRACTS

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
See below

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
DERWENT WPAT; Chemical Abstracts CASM; MEDLINE; STN Genbank, Chemical Abstracts

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	WO 94/22486 (THOMAS JEFFERSON UNIVERSITY) 13 October 1994; see whole document.	14-16, 23-24
X,P Y,P	Batch, J.A. et al. (1994) Localization of Messenger ribonucleic acid for insulin-like growth factor binding proteins in human skin by in situ hybridization, Journal of Clinical Endocrinology and Metabolism, vol. 79, no. 5 pages 1444-1449, November 1994	23-24, 26-29 1-29
Y	Cohick, W.S. and Clemmons, D.R. (1993) Regulation of IGFBP secretion and modulation of cell growth in MDBK cells, Growth Regulation, vol. 3, no. 1, pages 20-23, March 1993.	23-24, 26-29

[X] Further documents are listed in the continuation of Box C

[ ] See patent family annex

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"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
8 September 1995	26 SEPTEMBER 1995
Name and mailing address of the ISA/AU AUSTRALIAN INDUSTRIAL PROPERTY ORGANISATION PO BOX 200 WODEN ACT 2606 AUSTRALIA Facsimile No.: (06) 285 3929	Authorized officer D. HENNESSY Telephone No.: (06) 283 2255

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00410

C (Continuation) **DOCUMENTS CONSIDERED TO BE RELEVANT**

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Singh, P. et al. (1994) Episomal expression of sense and antisense insulin-like growth factor (IGF) - binding protein-4 complementary DNA alters the mitogenic response of a human colon cancer cell line (HT-29) by mechanisms that are indepedant of and dependant upon IGF-I, Cancer Research, vol. 54, pages 6563-6570, 15 December 1994.	23-24, 26-29
Y,P	Long, L. et al. (1995) Loss of metastatic phenotype in murine carcinoma cells expressing an antisense RNA to the insulin-like growth factor receptor, Cancer Research, vol. 55, pages 1006-1009, 1 March 1995.	14-16, 23-24, 26-29
X,P	Resnicoff, M. et al .(1994) Growth inhibition of human melanoma cells in nude mice by antisense strategies to the type 1 insulin-like growth factor receptor, Cancer research, vol. 54, pages 4848-4850, 15 September 1994.	14-16, 23-24, 26-29
Y,P	Shapiro, D.N. et al. (1994) Antisense-mediated reduction in insulin-like growth factor-I receptor expression suppresses the malignant phenotype of a human alvcolar rhabdomyosarcoma, J. Clin. Invest. Volume 94, pages 1235-1242, September 1994.	14-16, 23-24, 26-29
P,Y	WO 94/23034 (Cedars-Sinai Medical Center) 13 October 1994; see "Background of the Invention" in particular.	1-29

INTERNATIONAL SEARCH REPORT

International Application No.
PCT/AU 95/00410

Box 1 Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☒ Claims Nos.: 12, 14-16, 21, 29
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

Under rule 33.3(b) the claims relate to speculative matter and the specific search would be financially unreasonable.

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a)

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.